

EVALUATION OF NITRITE-INDUCED OXIDATIVE MODIFICATION OF HEMOGLOBIN AND THE POSSIBILITY OF ITS REGULATION BY SODIUM SELENITE BY RAMAN MICROSCOPY

T.M. HUSEYNOV, M.Z. DADASHOV, S.Y. HUSEYNOVA

Institute of Biophysics of Azerbaijan NAS

117, Zahid Khalilov str., Baku, AZ 1141

tokhus@mail.ru

The method of microscopic recombination scattering was used to assess the conformational changes in hemoglobin under the influence of sodium nitrite (NaNO_2) taken in small (0-0.35 μM) and moderate (0.35-0.70 μM) final concentrations and sodium selenite (Na_2SeO_3 - 5 μM) in incubation medium containing human erythrocytes (incubation time 30 min). Under the influence of NaNO_2 , there is a dose-dependent decrease (up to 60%) in HbO_2 content, an increase ($\approx 25\%$) in the ability of hemoglobin to bind ligands and an increase ($\approx 12\%$) in the ability of hemoglobin to release ligands (O_2), as well as an increase (up to 13%) in the severity of symmetric and asymmetric pyrolic hematoporphyrin rings. Sodium selenite reduces the oxidative effects of sodium nitrite decreasing the increase in MetHb accumulation and a decrease of hemoglobin saturation by oxygen.

Keywords: sodium nitrite, sodium selenite, hemoglobin, erythrocytes, Raman scattering.

PACS:

1. INTRODUCTION

In the last 20-30 years, there has been an increased interest in the participation of nitrogen-containing compounds in human life, in particular nitrites and its main metabolite which is nitrogen oxide. One of the important aspects of this interest is their toxicity due to high emissions of nitrogen oxides into the biosphere by industrial enterprises and transport, nitrites in agricultural production, nitrites in the food and pharmaceutical industry [1-4]. The toxicity of nitrites and its main metabolite, nitric oxide, is due to their ability to influence the oxidative status of hemoglobin (Hb), which transports O_2 , CO_2 , and NO [1,2].

Relatively recently, it was found that NO takes part in allosteric transformations of Hb [5], despite the fact that NO has a significantly higher affinity constant for hemoporphyrin ($\text{Fe} + 2$) than O_2 , and thus competes with oxygen for binding to hemoglobin [3, 6,7], which determines its influence on the hemoglobin conformation and on its affinity for oxygen. Nitric oxide is incorporated into hemoporphyrin, forming nitrosyl hemoglobin (HbNO), and cysteyl residues of the α and β polypeptide chains of hemoglobin (nitrosothiols) [1]. Of particular interest is the incorporation of NO into 93 βcysHb (SNOHb (β 93)) due to the proximity of this fragment to heme, which allows the transition from heme (HbNO position) to this position and vice versa [1,7]. The binding of NO to the SH groups of the α and β chains of Hb increases the affinity of NO for hemoglobin and prevents the transition to the T-conformational state, which has a low affinity of Hb for oxygen. During the transition from the R-state to the T-state, NO is released from the SH-residue 93 β of cysteine into the free state, or passes into hemoporphyrin of hemoglobin (HbNO) [7]. Thus, hemoglobin acts as a utilizer of excess NO ; is oxidized to metHb and possibly to deeper forms of oxidation (1-6). In addition, an excess of NO (or

nitrite) coming from outside or as a result of endogenous synthesis can lead to a violation of the prooxidant-oxidative equilibrium (oxidative stress) in erythrocytes with the formation of reactive oxygen and nitrogen species (peroxynitrite) [8]. Erythrocytes have an effective antioxidant (AO) system, which includes superoxide dismutase (SOD), catalase, glutathione peroxidase (GP), peroxiredoxin-2 (Prx-2), glutathione reductase, etc. [9]. One of the important components of the AO system of erythrocytes is selenium, which is included in the active center of GP, as well as nonspecifically included in hemoglobin, replacing sulfur in sulfur-containing amino acid residues [10,11] and affecting the oxidative resistance of hemoglobin [12]. These considerations suggest that the toxic effect of nitrite or NO on hemoglobin and on erythrocytes in general can be limited by the use of selenium in the form of sodium selenite, which has an active metabolism in erythrocytes: it easily penetrates into the cytosol, initially it is almost completely incorporated into hemoglobin by a complex redox mechanism, being reduced to selenide, it leaves through the AE1 anion exchanger, partially remaining in hemoglobin, essentially in certain vacant localization sites, as NO in polypeptide chains ($\text{SNO}\beta\text{cys}$) [13, 14]. It should be noted that if the 93 βcys amino acid residue provides AO protection of hemoglobin [15] protecting hemoporphyrin, then it can be assumed that its selenium analog can do this more efficiently due to its greater reactivity (electronegativity) [16].

It follows that the toxic effect of nitrites or nitric oxide in the form of oxidative effects on hemoglobin and erythrocytes can be weakened by selenium when the erythrocytes (hemoglobin) are enriched with selenite. From the above, it can be seen that both nitrites and selenites, when penetrating into erythrocytes, are actively metabolized and incorporated into hemoglobin, exerting a certain effect on it, which can affect its functional activity and, first

of all, its ability to bind with oxygen. This is closely related to its conformational state.

In this regard, we used the method of microscopic recombination scattering (MCR) to assess possible conformational changes of hemoglobin caused by nitrite and selenite exposure, which allows non-invasively, by the characteristic frequencies of the Raman spectrum, to trace the effects of induced exposure: sodium nitrite and selenite [17,18,19,20].

2. MATERIALS AND METHODS

In the experiments, sodium chloride, sodium nitrite, monosubstituted and disubstituted sodium phosphate monosubstituted and disubstituted were used (CP quality, Russia).

In experiments *in vitro*, donor blood was used, taken from the cubital vein into tubes with heparin (20 units / ml of blood). The main object of the study was human erythrocytes and Hb. Separation of blood plasma from erythrocytes was performed by centrifugation (800 g for 15 min). To obtain a suspension of erythrocytes, the erythrocyte sediment was washed three times in a tenfold volume of sodium phosphate buffer solution (SPB) (10 mM SPB pH 7.4; 0.15 mM NaCl), centrifuged at 800 g for 15 min, followed by removal of the supernatant. Hemolysis of erythrocytes was achieved by diluting the erythrocyte mass with distilled water in a ratio of 1: 9, followed by freezing, thawing and centrifugation at 10000 g. The nitrite effect was carried out with sodium nitrite NaNO₂ in four final concentrations (small - 0.07, 0.15 mM and moderate -0.35, 0.70 mM), exposure time from 0 to 30 minutes, at 37 ° C. These low toxicity doses were taken to track the oxidative process of Hb caused by nitrite. Raman spectra of Hb were recorded using a microscopic system 3D-confocal Raman spectrometer Nanofinder 30 (Japan). To excite the Raman spectra, we used a laser with a wavelength of 532 nm, radiation power 10 mW, objective 50 x. The presence of bands in the Raman spectrum reflects the structural and functional state of the studied material - hemoglobin. In particular, the state of the iron atom (Fe) and its ligands (O₂, NO, CO₂, etc.) that bind to it, affecting the state of the structure of the tetrapyrrole cycle of hemoporphyrin (porphyrin ring) of hemoglobin. To analyze the conformational changes and O₂-binding properties of hemoglobin, we used the characteristic bands of the Raman spectra of erythrocytes 1172, 1355, 1548-1552, 1580-1588, 1618-1668 [17, 18, 19].

Sodium selenite, which has a high permeability through the erythrocyte membrane and an active metabolism in them, was used as a possible antioxidant affecting oxidative processes. The final concentration in the incubation medium was selected based on previous experiments. In particular, before using sodium selenite as an antioxidant, need to take into account that selenite in excess, along with the AO effect, can enhance lipid peroxidation, reduce the content of NADPH, GSH, oxidizing the SH-groups of hemoglobin and other proteins, and ultimately

provoke hemolysis of erythrocytes [11]. In this regard, it was necessary to choose its optimal concentration range of antioxidant effects on erythrocytes. In our early works, it was found that sodium selenite, with a final concentration in the incubation medium of up to 10 μM (incubation period 0-30 min), does not have a prooxidative effect, does not oxidize erythrocyte GSH, and at final concentrations in the range of 1.0-10 μM, it has a certain AO effect: it slows down the accumulation of MetHb, slightly increases the activity of catalase and GP, and has no effect on lipid peroxidation [21]. Based on this, the final concentration of sodium selenite 5 μM was chosen as the optimal one.

To assess the ratios of nitrite-oxidized forms of Hb in erythrocytes, two series of experiments were carried out with the incubation times of the samples (30 min) at 25 ° C. Each series included one control and one prototype. The control sample contained 2.0 ml of erythrocyte suspension and 0.2 ml of buffered saline solution, and the test samples - 2.0 ml of erythrocyte suspension and 0.2 ml of NaNO₂ with different final concentrations.

The accumulation of metHb was assessed using semiempirical formulas [22]. The hemoglobin content was assessed by the standard cyanide method.

Statistical processing of the obtained results was carried out using the t-test at a significance level of p <0.05 [23] using the Microsoftexcel 2010 software package.

3. RESULTS AND THEIR DISCUSSION

Figure 1 shows the Raman spectrum of erythrocytes, which essentially reflect the spectrum of hematoporphyrin (600-1800 cm⁻¹). The family of bands (1375-1355 cm⁻¹) is the frequency region responsible for the vibrations of C-N bonds, the position of which depends on the oxidation state of the iron atom, while the frequency section 1650-1562 cm⁻¹ is responsible for the vibrations of C=C bonds, whose positions depend on the spin of the iron ion. The binding of such ligands as O₂ and NO causes a conformational rearrangement, which is associated with a change in spin. The content (concentration) of HbO₂ (zero spin charge) is estimated from the ratio of the intensity of the bands (I1375 / I1355 + I1375). The formation of a complex of hematoporphyrin with NO is possible in two variants: one is realized without breaking the bond on histidine of the protein part of Hb (ratio I1620 / I1580), in the second variant, this bond is broken (ratio I1658 / I1580), which means the ability of NO to regulate the release of oxygen from hemoglobin, i.e. its transition from a low-spin state to a high-spin state (5/2). In the case of direct oxidation of hemoglobin by sodium nitrite, π-electrons are "pumped" from the iron ions (Fe²⁺ → Fe³⁺) to the orbital of nitrogen oxides, reducing them to nitrates, which is reflected in the vibrational component of the binding energy (shift 1379-1372 cm⁻¹), as well as a decrease in the maximum at 1357 cm⁻¹ (characterizes the growth of MetHb) [17, 18]. The substitution of NO for O₂ in the Hb porphyrin macrocycle ultimately

leads to a change in the position of the iron atom (Fe^{2+}) with respect to the plane of the macrocycle, which is reflected in the spin state, i.e. allows to judge the degree of conformational rearrangement [17, 18, 19].

It is known that nitrites, both in isolated erythrocytes and in vivo, depending on their dose, oxidize hemoglobin to different oxidation states. This process is accompanied by a decrease in the level of oxyhemoglobin (HbO_2) [1,6,7]. In our previous works, it was shown that even low final concentrations of NaNO_2

(0.007–0.070 mM) in the incubation medium already in the first 15–30 min can significantly affect the accumulation of methemoglobin, without accelerating the development of LPO, rather, on the contrary, inhibiting it [24, 25]. In experiments using sodium selenite as an antioxidant, it was also found that it can inhibit the accumulation of MetHb and the development of LPO (moderate doses of NaNO_2) [24,25].

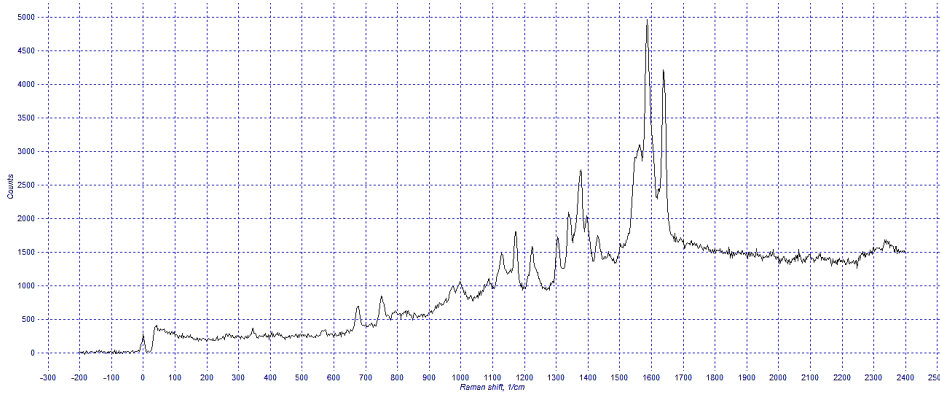


Fig 1. Raman spectra of hemoglobin in human erythrocytes diluted 1/50 in buffered saline (pH 7.4; 25 ° C, laser $\lambda = 532$ nm).

Figure 2 shows the results of experiments on the induced oxidation of hemoglobin with low and moderate doses of sodium nitrite and the inhibitory effect of sodium selenite taken at a dose of 5 μM (30 min incubation). The accumulation of MetHb is due to the decrease in HbO_2 , which can be traced using Raman spectroscopy, which, in

contrast to optical spectroscopy, is distinguished by higher selectivity and sensitivity. In particular, Fig. 3 shows the estimated relative intensities of the peaks responsible for the state of heme groups at 677 cm^{-1} , 1375 cm^{-1} , 1580 cm^{-1} , 1640 cm^{-1} .

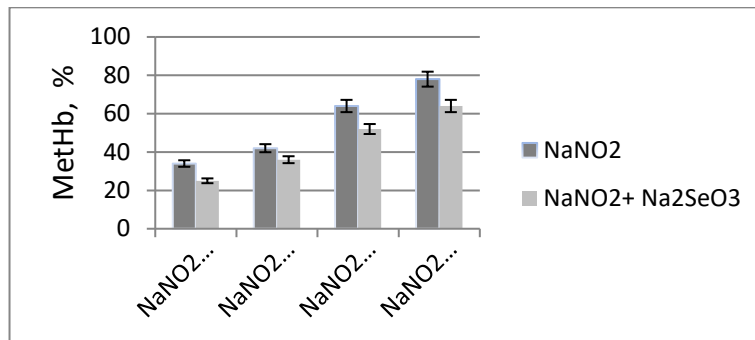


Fig 2. Accumulation of MetHb at various final concentrations of NaNO_2 and participation of Na_2SeO_3 (5 μM) (25 ° C, 10mM SPB, pH 7.4; 30 min)

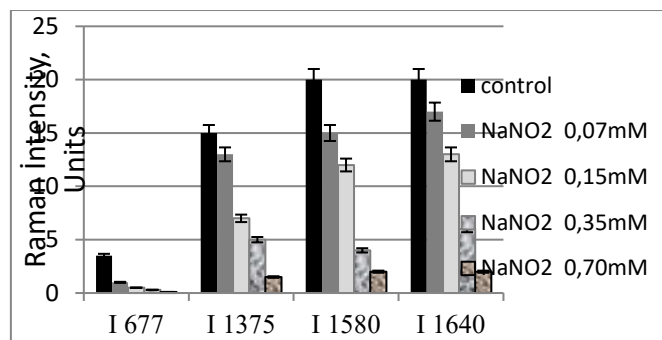


Fig 3. Intensity of Raman spectra of erythrocytes incubated with NaNO_2 (0.07, 0.15, 0.35, 0.70 mM) (25 ° C, 10mM SPB, pH 7.4; 30 min)

Table

Parameters of RAMAN spectroscopy of hemoglobin of erythrocytes treated with sodium nitrite at various concentrations (small 0-0.35 and moderate 0.35-0.70 mM) in the presence of sodium selenite (5 μM). (25 °C, 10mM SPB, pH 7.4; 30 min).

Index	Experimental groups											
	kontrol	NaNO ₂				NaNO ₂ + Na ₂ SeO ₃ (5 μM)						
		0,07 mM	0,15 mM	0,35 mM	0,70 mM	0,00 mM	0,07mM	0,15 mM	0,35 mM	0,70 mM		
The proportion of oxyhemoglobin in erythrocytes (I ₁₃₇₅ /I ₁₃₅₅ +I ₁₃₇₅)	0,73 ± 0,08	0,62 ± 0,04 *	0,55 ± 0,05 *	0,41 ± 0,04 *	0,75±0,05	0,70±0,07	0,67±0,04 *	0,61±0,05 **	0,53±0,06 **			
The ability of hemoglobin to bind ligands (I ₁₃₅₅ /I ₁₅₅₀)	0,68± 0,03	0,77 ± 0,05 *	0,83 ± 0,07 *	0,90 ± 0,08 **	0,66±0,06	0,70 ± 0,05	0,72 ± 0,06	0,77 ± 0,05 *	0,85 ± 0,05 **			
The ability of hemoglobin to separate ligands (I ₁₃₇₅ /I ₁₅₈₀)	0,71 ± 0,06	0,75 ± 0,06	0,78 ± 0,05 *	0,81 ± 0,06 *	0,71 ± 0,08	0,72±0,06	0,73 ± 0,05	0,72±0,04	0,72±0,06			
The affinity of hemoglobin for oxygen (I ₁₃₅₅ /I ₁₅₅₀)/(I ₁₃₇₅ /I ₁₅₈₀)	1,04 ± 0,05	0,93 ± 0,07 *	0,88 ± 0,12 *	0,81 ± 0,15 *	1,05 ± 0,07	1,02±0,12	0,97 ± 0,11 *	0,90±0,10	0,85±0,15			
The severity of symmetric and asymmetric vibrations of pyrrole rings (I ₁₃₇₅ /I ₁₁₇₂)	1,20 ± 0,04	1,29± 0,09 *	1,35 ± 0,10 *	1,41 ± 0,11 **	1,21 ± 0,17	1,22 ± 0,06	1,24 ± 0,08	1,30 ± 0,10 *	1,33±0,11 **			
Fraction of complexes of hemoglobin with NO in the presence of a bond between the Fe ²⁺ atom and globin (I ₁₆₁₈ /I ₁₅₈₀)	0,22±0,02	0,16 ± 0,03 **	0,12 ± 0,04 **	0,08 ± 0,02 **	0,22±0,02	0,20 ± 0,02	0,18 ± 0,03 *	0,15±0,05 *	0,21±0,04			
Fraction of complexes of hemoglobin with NO in the absence of a bond between the Fe ²⁺ atom and globin (I ₁₆₆₉ /I ₁₅₈₀)	0,41 ± 0,02	0,45 ± 0,03	0,47 ± 0,03 *	0,55 ± 0,05 **	0,40±0,05	0,41±0,03	0,42±0,04	0,45±0,05 *	0,43±0,06 *			

Note * $p < 0.05$, ** $p < 0.01$

Fig. 3 shows that as the final concentrations NaNO_2 increase, the peaks at 677 cm^{-1} , 1375 cm^{-1} , 1580 cm^{-1} , 1640 cm^{-1} decrease or even disappear (a band characterizing the bond with hemoglobin). The most sensitive to the spin state is the 1375 band, which characterizes the degree of decrease in the HbO_2 saturation.

Table shows the main parameters of Raman scattering spectroscopy of human erythrocytes incubated in a medium containing sodium nitrite in various final concentrations from 0 to 0.70 mM and sodium selenite with a final concentration of $5\text{ }\mu\text{M}$ for 30 min ($25\text{ }^\circ\text{C}$, 10 mM sodium phosphate buffer, 0.15 M NaCl). It can be seen from it that the treatment with NaNO_2 leads to:

1. A dose-dependent decrease (to 60%) of the HbO_2 content, which is estimated from the ratio of the intensity of the peaks $I_{1375} / I_{1355} + I_{1375}$.
2. A noticeable increase ($\approx 25\%$) in the ability of hemoglobin to bind ligands, including O_2 (I_{1355} / I_{1580}).
3. Some increase ($\approx 12\%$) in the ability of hemoglobin to give ligands, including oxygen (I_{1375} / I_{1580}).
4. Certain multidirectional change in the affinity of hemoglobin for oxygen, estimated by the ratio $(I_{1355} / I_{1550}) / (I_{1350} / I_{1580})$.
5. A noticeable increase in the severity of symmetry and asymmetry of vibrations of the pyrrole rings of hemoglobin in isolated erythrocytes (I_{1375} / I_{1172}).
6. A significant decrease in the proportion of hemoglobin complexes with NO (up to 50%, depending on the final concentration of NaNO_2) in the presence of a bond between the Fe^{2+} atom and globin (I_{1618} / I_{1580}).
7. Some increase in the proportion of complexes of hemoglobin with NO, with the separation of the bond of the Fe^{2+} atom and globulin, which changes little depending on the concentration of NaNO_2 (I_{1618} / I_{1580}).
8. Sodium selenite ($5\text{ }\mu\text{M}$) introduced into the incubation medium has a clear AO effect, reducing the effects of the oxidative effect of NaNO_2 , i.e. reduces the effects of conformational rearrangements. This is especially noticeable in the effect of inhibition of reducing the saturation of hemoglobin with oxygen (HbO_2). This AO effect is hardly noticeable in the range of low concentrations (0.07 - 0.35 mM), but above 0.35 mM, i.e. at moderate final concentrations of NaNO_2 , it is noticeably pronounced.

It is also important to note here that sodium selenite smoothes not only a decrease in the HbO_2 level and, accordingly, an increase in MetHb, but also changes in other parameters of conformational rearrangements caused by nitrites. One of the possible mechanisms of this effect is the very fact of the inclusion of selenium in the globular part of hemoglobin at position 93 β cys, replacing sulfur, thereby affecting the affinity of hemoglobin for

oxygen under conditions of nitrite exposure, changes, in general, the electron density around heme [26].

Table 1 shows that sodium nitrite affects the conformation of hemoglobin and this rearrangement is the deeper, the greater the dose of NaNO_2 (final concentration). However, on the affinity of hemoglobin for oxygen, in the indicated final concentrations, NaNO_2 has a somewhat multidirectional effect: small doses (0.07-0.15 mM) can increase the affinity, and moderate doses (0.35-0.70 mM) can reduce. This seems to be due to the fact that the affinity of hemoglobin for oxygen is influenced by two opposite processes. Formed during nitrite exposure, NO is incorporated into heme with the formation of nitrosyl hemoglobin, contributing to the formation of five-coordinate nitrosyl iron (HbNO) by separation of the proximal ligation. This leads to the stabilization of the T-state, which provides an easier release of oxygen, i.e. a decrease in affinity [27], which takes place for moderate doses of NaNO_2 . On the other hand, by nitrosylation (inclusion of NO at the 93 β cysHb position), the formation of SNO-Hb increases the affinity of Hb for oxygen due to a change in the electron density [7].

It also follows from the ratio of the intensities of the peaks $I_{1375} : I_{1172}$ that sodium nitrite has a dose-dependent effect of increasing (up to 13%) the severity of symmetric and asymmetric vibrations of the pyrrole rings of hematoporphyrin. This effect can be associated with an increase in the proportion of membrane-bound hemoglobin in isolated erythrocytes [28]. Our earlier results also indicate this [25,29,30], i.e. an increase in the accumulation of membrane-bound hemoglobin, which penetrating through the AE1 anion exchanger into the erythrocyte membrane can stimulate the LPO process [28].

The totality of the results obtained allows us to draw certain conclusions: Raman microscopy makes it possible to determine with high sensitivity the onset of conformational rearrangements in hemoglobin caused by the action of sodium nitrite. This can be seen: 1) by the example of fixing multidirectional changes in the affinity of Hb for oxygen, depending on the "dose" of the effect of sodium nitrite on erythrocytes (hemoglobin); 2) depending on the final concentrations of sodium nitrite, a rupture of the bond between hemoglobin and an iron atom and a change in the valence of an iron atom ($\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$) may occur; 3) by the effect on the oscillatory processes in the pyrrole rings of hematoporphyrin, which can give information about the accumulation of membrane-bound hemoglobin, which stimulates the development of oxidative processes in erythrocytes; 4) Included selenium from sodium selenite smoothes out the conformational effects induced by sodium nitrite, while weakening oxidative processes on Hb, reducing the accumulation of MetHb and reducing the proportion of membrane-bound hemoglobin.

[1] A.W. DeMartino, D.B Kim-Shapiro, R.P. Patel, M.T. Gladwin. Nitrite and Nitrate Chemical

Biology and Signalling. Br J Pharmacol. 2019;176(2):228-245. doi: 10.1111/bph.14484.

- [2] E.I. Nasybullina, O.V. Kosmachevskaya, A.F. Topunov. Influence of nitric oxide metabolites on the formation of membrane-bound hemoglobin under carbonyl stress. "Fundamentals of Biotechnology" RAS, Moscow, Russia. 2018; 4: 93–104. doi: 10.17076 / them814.
- [3] A.F. Vanin. Dinitrosyl iron complexes with thiol-containing ligands as a «working form» of endogenous nitric oxide, nitric Oxide Biol. Chem. 2016; 54: 15-29.
- [4] A.K. Martusevich, A.G. Solovieva, S.P. Peretyagin, A.F. Vanin. Comparative analysis of the effect of free and deposited NO on the state of pro- and antioxidant systems in the blood. "Biophysics" ". 2015; 60 (2): 348-354.
- [5] Duke researchers discover central role of Nitric Oxide in hemoglobin action. Duke Medicine News and Communication. 3 Nov. 2004. dukehealth.org
- [6] M.K. Ruban, G.A. Vashanov, I.A. Lavrinenko. Oxygenation-induced structural and functional modifications of nitrosylated hemoglobin. VSU Bulletin, series: chemistry. biology. pharmacy, 2010;1:56-61.
- [7] A.I. Hobbs, M.T. Gladwin, R.P. Patel, D.L.H. Williams, A.R. Butler. Haemoglobin: NO transporter, NO inactivator or none of the above. TRENDS in Pharmacological Science. 2002; 23: 406–411.
- [8] P.K. Maurya, P. Kumar, P. Chandra. Biomarkers of oxidative stress in erythrocytes as a function of human age. World J Methodol. 2015; 5(4): 216–222. doi: 10.5662/wjm.v5.i4.216.
- [9] S. Rocha, D. Gomes, M. Lima, E. Bronze-da-Rocha, Santos-Silva A. Peroxiredoxin 2, glutathione peroxidase, and catalase in the cytosol and membrane of erythrocytes under H₂O₂-induced oxidative stress. Free Radic Res. 2015;49(8):990-1003. doi: 10.3109/10715762.2015.1028402.
- [10] M.A. Beilstein, P.D. Whanger. Distribution of selenium and glutathione peroxidase in blood fraction from humans, rhesus and a quere monkey, rats and sheep. J. Nutr; 1983; 113(11):2138-2146. doi:10.1093/jn/113.11.2138.
- [11] R.F. Burk, K.E. Hill. Regulation of Selenium Metabolism and Transport. Annu Rev Nutr 2015;35:109-34. doi: 10.1146/annurev-nutr-071714-034250.
- [12] T.M. Huseynov, F.R. Yakhyeva, R.T. Gulieva. Effect of selenium on the resistance of hemoglobin to photooxidative processes. Ukrainian Biochemical Journal. 2012; 84(2): 53-60.
- [13] K.T. Suzuki, Y. Shiobara, M. Itoh, M. Ohmichi. Selective uptake of selenite by red blood cells. Analyst. 1998;123(1):63-67. doi: 10.1039/a706230c.
- [14] M. Haratake, K. Fujimoto, M. Ono, M. Nakayama. Selenium binding to human hemoglobin via selenotrisulfide. Biochim Biophys Acta. 2005; 25;1723(1-3):215-220. doi:10.1016/j.bbagen.2005.02.002.
- [15] D.A. Vitturi, C.W. Sun, V.M. Harper. Antioxidant Functions for the Hemoglobin β93 Cysteine Residue in Erythrocytes and in the Vascular Compartment in Vivo. Free Radic Biol Med. 2013; 55:119-29. doi: 10.1016/j.freeradbiomed.2012.11.003.
- [16] U.M. Viswanathan, T. Burkholz and C. Jacob. Electrochemistry at the edge of reason: Chalcogen-based redox systems in biochemistry and drug design, Zeitschrift für Physikalische Chemie, 227(5): 691-706 (2013). doi.org/10.1524/zpch.2013.0321
- [17] G.V. Maksimov, N.A. Brazhe, A.I. Yusipovich, E.Yu. Parshina, O.V. Rodnenkov, A.B. Rubin, G.G. Levin, V.A. Bykov. The use of nanoparticles to study the conformations of the approximate hemoglobin. Biophysics, 2011; 56(6):1099-1104
- [18] P. Ivo, J. Terner, R.N. Pittman, E. Proffitt and K.R. Ward. Measurement of hemoglobin oxygen saturation using Raman microspectroscopy and 532-nm excitation. J Appl Physiol. 2008; 104: 1809–1817. doi:10.1152/japplphysiol.00025.2008.
- [19] M.F. Zhu, Y.Y. Huang, Z.Y. Guo, Z.F. Zhuang, S.H. Liu. Detection of methemoglobin in whole blood based on confocal micro-Raman spectroscopy and multivariate statistical techniques. Scanning 2014; 36: 471–478. doi: 10.1002/sca.21143
- [20] S.Y. Huseynova, T.M. Huseynov, M.Z. Dadashov. Oxidative modification of hemoglobin by nitrites and the protective role of selenium. Possibilities of Raman spectroscopy. Azerbaijan journal of physics. Baku, 2015; 21(03): 16-20.
- [21] S.Y. Huseynova. Oxidative metabolism of sodium selenite in isolated human erythrocytes in vitro. Biomedicine (Baku).2019;17(3):18-23. doi: 10.24411/1815-3917-2019-10018
- [22] J. Szebeni, C.C. Winterbourn, R.W. Carrell. Oxidative interactions between haemoglobin and membrane lipid. A liposome model. Biochem. J. 1984. V. 220. No 3. P. 685–692
- [23] G.F. Lakin. Biometriya. Moscow, 1990; 352 p.
- [24] S.Y. Huseynova, R.T. Gulieva, M.Z. Dadashov, A.I. Jafarov, F.R. Yakhyeva, T.M. Guseinov. Oxidative modification of hemoglobin of isolated erythrocytes in an incubation medium containing donors of nitric oxide and sodium selenite. Bulletin of the Novosibirsk State Pedagogical University. 2016; 6(5):207-215.
- [25] S.Y. Huseynova, T.M. Huseynov, M.Z. Dadashov. Oxidative modification of erythrocytes with moderate doses of sodium nitrite in in vitro experiments, Medical biophysics and biochemical chemistry. 2018;(3)1:189-195.
- [26] H. Kosaka. Nitric oxide and hemoglobin interactions in the vasculature. Biochim. Biophys. Acta . 1999; 1411: 370–377.
- [27] M.C. Garel, C. Domenget, J. Caburi-Martin, C. Prehu, F. Galacteros, Y. Beuzard. Covalent binding of glutathione to hemoglobin. I. Inhibition of hemoglobin S polymerization. J. Biol. Chem. 1986; 261:14704–14709.

- [28] *N.A. Brazhe, S. Abdali, R.A. Brazhe, O.G. Luneva et. al* New insight into erythrocyte through in vivo surface-enhanced raman spectroscopy *Biophys J.* 2009; 97(12): 3206–3214. doi: 10.1016/j.bpj.2009.09.029
- [29] *S.Y. Huseynova, T.M. Huseynov, M.Z. Dadashov.* Evaluation of nitrite-induced oxidative modification of hemoglobin by Raman spectroscopy. Abstracts of the International Scientific Conference dedicated to the 90th anniversary of the National Academy of Sciences of Belarus and the 45th anniversary of the Institute of Biophysics and Cell Engineering, Belarus, Minsk, June 27-29, 2018 g, page 149.
- [30] *S.Y. Huseynova.* Membrane-bound hemoglobin as an indicator of nitric toxicity of erythrocytes. *Azerbaijan chemical journal* 2019, 2020 (2) : 67-73. doi.org/10.32737/0005-2531-2020-2-67-73.

Received:17.11.2020