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Kinetics and Mechanism of Hemoglobin Oxidation by Nitroethane

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Abstract—In the present paper the process of oxyhemoglobin oxidation by nitroethane has been investigated. The main process is accompanied with numerous side reactions including oxidative denitration of nitroethane resulting in the generation of acetaldehyde and 1,1-dinitroethane. The latter product is formed under the action of nitrite ion which is the product of oxidative denitration of nitroethane. The chain radical mechanism of methemoglobin generation is proposed. The reaction of oxyhemoglobin with nitroethane is regarded as initiated autooxidation of oxyhemoglobin.

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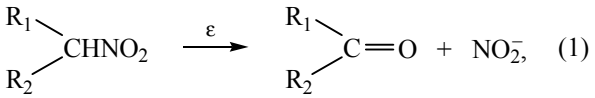
Nitro compounds are traditionally treated as hemoglobin oxidizing agents [1–5]. The ability of methemoglobin production by aromatic nitro compounds, namely, nitrobenzene, dinitrobenzene [6], trinitrotoluene [7], picric acid [8], nitroanilines [9], tetryl [10], nitrobenzoic acids derivatives [11–13] have been thoroughly investigated. Aliphatic nitro compounds are also known as hemoglobin oxidizers. The oxidation of hemoglobin by α-halo-α-nitroethanes was investigated in detail [14–15] and this reaction was shown to have chain radical mechanism accompanied with the degenerated chain branching [16–19]. Based on the above data, the nitroethane ability to oxidize hemoglobin was anticipated. Nitroalkanes are highly reactive organic compounds undergoing various chemical transformations [20–25] both *in vitro* [26–28] and *in vivo* [29–30]. Usually in mammalian organisms nitroalkanes undergo oxidative denitration producing carbonyl compounds **I** [31–37].



(1)

where ε is an enzyme (FAD-dependent dehydrogenaze isolated from *Fusarium oxysporum*).

Such reaction also takes place in the cells of plants and microorganisms [38–40]. The generation of nitrite ion in the reaction of oxidative denitration *in vitro* takes place both spontaneously [41–42] and under the action of enzymes isolated from biological objects [43]. The methemoglobin production under the action of inorganic nitrites has been actively investigated by numerous groups of chemists and biochemists [44–61]. The process of hemoglobin oxidation by nitrogen dioxide [62–63] and peroxinitrite ion ($ONOO^-$) [64] possibly generated in the reaction of hemoglobin oxidation by nitrite ion [44] are also under investigation.

Minding the probable nitroalkane denitration [reaction (1)], the process of methemoglobin production under the action of nitroethane should be rather close to hemoglobin oxidation by inorganic nitrites. A lot of research of the process of methemoglobin production under the action of nitrite ion proved its chain mechanism and showed that it was initiated by electron transfer from nitrite ion to the oxygenated form of hemoglobin [49–51, 56, 59, 65].

Electron donating properties of nitrocarbanions are well-known and a lot of chemical reactions in which nitroalkane anions act as electron donating agents are described [66]. So *a priori* it may be presumed that methemoglobin production *in vitro* under the action of nitroethane would take place, most probable under the conditions of nitroalkanes deprotonation. But the detailed mechanism can be established only by kinetic experiments.

The nitrite ion generated according to reaction (1) further takes part in the oxyhemoglobin reduction accompanied by the production of nitrogen dioxide, possibly peroxynitrite ion, and active oxygen species. Because a plethora of reactive particles are generated in the reaction, the process of methemoglobin production may be rather intricate and a thorough systematic investigation is needed to elucidate its details.

We investigated the kinetics of methemoglobin production under the action of nitroethane in pH range 6.6–8.35 which warrants the presence of the nitrocarbanion (pK_a = 8.5 [67]). The reaction took place in an aqueous phosphate buffer solution. Hemoglobin concentration was in the interval from 1.3×10^{-5} to 2.1×10^{-4} hem l^{-1} and nitroethane concentration was in the interval 0.05–0.26 M.

In our kinetic experiments the method of initial rates was used. The most probable way of methemoglobin production in the present system includes the initiation of the process through one electron oxidation of nitroethane anion by oxyhemoglobin molecule [reaction (2)] with further fast fragmentation of the so produced radical anion according to scheme (3):

$$CH_3CH{=}NOO^- + HbO_2 \rightarrow [HbO_2]^{\cdot-} + CH_3\dot{C}HNO_2, \quad (2)$$

$$[HbO_2]^{\cdot-} \rightarrow Hb + O_2^{\cdot-}, \quad (3)$$

Hb is hemoglobin.

Minding the presence in the reaction system of the non-ionized form of nitroethane, the latter may play the role of a proton donating agent and may catalyze the oxyhemoglobin radical anion decomposition [Eq. (4)]:

$$[HbO_2]^{\cdot-} + CH_3CH_2NO_2 \rightarrow Hb + CH_3CH{=}NOO^- + HO_2^{\cdot}. \quad (4)$$

In this case the generation of free radical species may be described by the overall process shown below [Eq. (5)], combining equations (2) and (4):

$$HbO_2 + CH_3CH_2NO_2 \rightarrow Hb + CH_3\dot{C}HNO_2 + HO_2^{\cdot}. \quad (5)$$

Two reactive free radicals are generated in this reaction but $HO_2^{\cdot}$ radical is more acidic and so its ionization takes place (pK_a = 4.88 [68]) [Eq. (6)]:

$$HO_2^{\cdot} + B: \rightleftarrows BH^+ + O_2^{\cdot-}, \quad (6)$$

where B stands for a base.

Under the experimental conditions the protolytic equilibrium between hydroperoxide radical and superoxide radical anion is shifted to the right though about 0.5–1% of superoxide radical ion remains protonated. The oxidation potential of $HO_2^{\cdot}$ towards biological substrates is much higher in comparison with $O_2^{\cdot-}$ which has been experimentally proved in the reaction of arachidonic acid oxidation [69]. The rate constants of arachidonic acid oxidation under the action of $HO_2^{\cdot}$ and $O_2^{\cdot-}$ are equal to 3×10^3 and 0.1 l mol^{-1} s^{-1}, respectively. In this case, taking into account the chain mechanism of the process, even negligible amount of hydroperoxide radical is enough to effect the methemoglobin production. Besides, considering extremely high rates of electron transfer processes, the oxidation of heme iron may take place simultaneously with the hydroperoxide radical generation, leaving behind the process of its acidic dissociation. The subsequent oxidation of heme iron evidently takes place with the participation of hydroperoxy radical according to scheme (7) given below:

$$Hb(Fe^{+2})O_2 + HO_2^{\cdot} \rightarrow MetHb(Fe^{+3}) + HO_2^- + O_2. \quad (7)$$

The so produced hydroperoxide anion is protonated according to Eq. (8), because the hydroperoxide pK_a value equals to 11.58 [70] and the reactivity of hydroperoxide anion as the proton donating agent is rather weak. So the involvement of this species into the electron transfer process at the moment of its production probably does not occur. The generated hydrogen peroxide further participates in hemic iron oxidation according to Eq. (9).

$$HO_2^- + H^+ \rightleftarrows H_2O_2, \quad (8)$$

$$H_2O_2 + Hb(Fe^{+2})O_2 \rightleftarrows {}^{\cdot}OH + OH^- + MetHb(Fe^{+3}) + O_2, \quad (9)$$

$$H_2O_2 + {}^{\cdot}OH \rightarrow HO_2^{\cdot} + H_2O. \quad (10)$$

The highly reactive radical ${}^{\cdot}OH$ generated in reaction (9) is able to interact with hydrogen peroxide according to the proposed scheme of the process regenerating $HO_2^{\cdot}$ radical (10), the latter being the chain leading radical of the reaction of hemoglobin oxidation to methemoglobin according to Eq. (7). So in the system under consideration a chain radical process is realized which is identical in many aspects to hemoglobin autooxidation [71–73]. Taking into account the stage of chain initiation involving the anion of nitroethane, this route of methemoglobin formation may be treated as the initiated oxidation described in [74–75].

The mode of chain termination is one of the main problems in establishing chain reactions mechanisms. In the framework of the proposed scheme the chain termination may take place both through quadratic

The influence of pH on the rate of methemoglobin production in the system HbO_2–nitroethane

pH	$[CH_2CH{=}NOO^-]\times10^2$ g-ion l^{-1}	$[CH_3CH_2NO_2]\times10^2$ M	$[H^+]\times10^8$ g-ion l^{-1}	$w\times10^7$ hem l^{-1} min^{-1}
7.30	0.9	12.6	5.02	2.58
7.48	1.3	12.2	3.30	2.50
7.70	1.8	11.6	2.00	2.24
7.98	3.1	10.3	1.05	2.05
8.05	3.5	9.9	0.89	2.00
8.35	6.3	7.1	0.44	1.67

recombination mechanism involving either $HO_2^{\cdot}$ or $^{\cdot}OH$ radicals or through the cross-recombination reaction of the same radicals. In order to definitely establish the mechanism of chain termination, the partial orders in the main reagents should be measured. In this work we have established the partial kinetic orders of the methemoglobin formation in the oxyhemoglobin–nitroethane reaction system with respect to the total concentrations of nitroethane and oxyhemoglobin.

At pH 7.2, 25°C; μ 0.1; and the total concentration of nitroethane 0.11 M the rate of the reaction correlates with oxyhemoglobin concentration: w(hem l^{-1} min^{-1}) = $(9\pm1)\times10^{-3}[HbO_2]\pm(0.21\pm0.09)\times10^{-6}$, r 0.9897, n 6. Hence, the reaction of methemoglobin production shows the first order in oxyhemoglobin concentration.

At pH 7.2, 25°C; μ 0.1; and the concentration of hemoglobin 3.8×10^{-5} hem l^{-1} the reaction has the order in nitroethane concentration equal to 0.5:

$$w(\text{hem}\ l^{-1}\ min^{-1}) = (9.3\pm0.4)\times10^{-7}([CH_3CH{=}NOO^-] + [CH_3CH_2NO_2])_{tot} \pm (0.39\pm0.09)\times10^{-7},$$
$$r\ 0.9913,\ n\ 6.$$

According to the established order of the reaction in hemoglobin concentration, the main chain termination route realizes through the recombination of $^{\cdot}OH$ and $HO_2^{\cdot}$ radicals [54, 71] [Eq. (11)]:

$$HO^{\cdot} + HO_2^{\cdot} \rightarrow H_2O + O_2. \qquad (11)$$

In the proposed scheme of oxyhemoglobin reaction with nitroethane all the stages are essentially the same as in the reaction of hemoglobin autooxidation [54, 71, 72] except for the initiation step [Eq. (5)], and the methemoglobin formation takes place according to reactions (5) and (7)–(11). So the methemoglobin production under the action of nitroethane realizes through the chain process with crossed chain termination that can be treated as initiated auto oxidation of oxyhemoglobin. In this case, according to the total scheme of free radicals generation, the rate of the methemoglobin accumulation should depend upon the concentration of the molecular form of nitroethane. To elucidate the catalytic role of the non-ionized form of nitroethane in the reaction, the dependence of the process rate on the proton concentration was investigated (see the table).

In the course of experiments a weak dependence of the methemoglobin accumulation rate on pH value of the medium was established. However, after the treatment of experimental data a fairly strict dependence of the process, rate upon the concentration of nitroethane was discovered.

$$w(\text{hem}\ l^{-1}\ min^{-1}) = (6.7\pm0.4)\times10^{-7}[CH_3CH_2NO_2]^{0.5} + (0.3\pm0.2)\times10^{-7},$$
$$r\ 0.9739,\ n\ 6.$$

According to the general regularities of chain processes, the reaction under investigation should have the first order in oxyhemoglobin concentration and the partial order equal to 0.5 in the molecular form of nitroethane. Just such partial orders in the reagents were established experimentally. The oxidation of hemoglobin is accompanied by a large number of side reactions. Parallel with the methemoglobin formation a radical oxidative denitration reaction takes place in the system whose mechanism was described by Russell in 1954 for 2-nitropropane [76]. In our case in the course of oxidative nitroethane denitration reactive nitroethyl [Eq. (12)] and ethyl [Eq. (13)] radicals are generated.

$$CH_3CH_2NO_2 + CH_3CHNO_2^- \rightarrow [CH_3CH_2NO_2]^{\cdot-} + CH_3\dot{C}HNO_2, \qquad (12)$$

$$[CH_3CH_2NO_2]^{\cdot-} \rightarrow CH_3\dot{C}H_2 + NO_2^-. \qquad (13)$$

The ethyl radical is further involved in the typical reactions of chain oxidation of hydrocarbons [77–83]

resulting in the formation of carbonyl compounds, namely, acetic aldehyde in our case. The overall scheme of carbonyl compounds formation is given below [Eq. (14)]:

$$CH_3CH_2^{\cdot} \xrightarrow{O_2} CH_3CH_2OO^{\cdot} \xrightarrow[-R^{\cdot}]{RH} CH_2CH_2OOH \xrightarrow[-OH^{\cdot}]{} CH_3CH_2O^{\cdot} \xrightarrow[-H_2O]{OH^{\cdot}} CH_3C(=O)H \quad (14)$$

This route of oxidative denitration correlates with the enzymatic denitration of nitroalkanes [Eq. (1)] [43] taking place under the action of FAD-dependent oxydoreductases isolated from *Hansenula mrakii* and *Fusarium oxysporum* cultivated on the media containing nitroethane additives [84–85].

Nitrite ion produced in the course of denitration reacts with oxyhemoglobin which is present primordially in the reaction medium. In such a manner the oxyhemoglobin should transform into methemoglobin both under the action of nitroethane and its denitration product, nitrite ion. In the course of the process the product of nitroethane oxidative denitration, acetic aldehyde, should accumulate; the latter was detected in the reaction medium in the amount of 2.3×10^{-3} M at pH 8.05, the concentration of nitroethane 13.5×10^{-2} M and the concentration of oxyhemoglobin 3.5×10^{-5} hem l^{-1}.

So the fact of oxidative denitration of nitroalkanes under the experimental conditions of oxyhemoglobin oxidation by nitroethane was experimentally confirmed.

The nitrite ion generated in the oxidative denitration reaction may be also involved into chain radical process of methemoglobin formation as it was previously described [19, 46–47]. But the kinetic curve of oxyhemoglobin oxidation by nitrite ion has a specific form that is typical for chain processes with degenerated chain branching [77] which is not observed in our case.

In the initial steps of the reaction of oxyhemoglobin with nitroethane according to the overall scheme of chain initiation [Eq. (5)] the hydroxy radical is generated in considerable amounts which resulted in the generation of radical $NO_2^{\cdot}$ and hydrogen peroxide according to reactions (15) and (8).

$$NO_2^- + HO_2^{\cdot} \rightarrow NO_2^{\cdot} + HO_2^-. \quad (15)$$

The route of the reaction can be described by reactions (16)–(18) proposed for the system oxyhemoglobin–nitrite ion at deep stages of conversion [49]:

$$NO_2^- + H_2O_2 \rightarrow OH^{\cdot} + NO_2^{\cdot} + OH^-, \quad (16)$$

$$NO_2^{\cdot} + Hb(Fe^{+2}) \rightarrow NO_2^- + MetHb(Fe^{+3}), \quad (17)$$

$$NO_2^- + {}^{\cdot}OH \rightarrow {}^-OH + NO_2^{\cdot}. \quad (18)$$

In this case the traditional S-type form of kinetic curves indicating methemoglobin production under the action of nitrite ion vanishes [49]. Analogously, when nitrite ion and hydrogen peroxide are simultaneously introduced into the reaction medium, the kinetic curves of methemoglobin formation loose their S-shape form. For the reaction of oxyhemoglobin oxidation with nitrite ion at deep stages of conversion the established mechanism of chain termination [49, 65] is described by Eq. (19):

$$NO_2^{\cdot} + NO_2^{\cdot} \rightarrow N_2O_4. \quad (19)$$

In our case the mechanism of chain termination is not clear because a lot of different free radicals are present in the reaction medium. So the processes of the oxidative denitration of nitroethane and methemoglobin formation are connected through co-metabolites.

It should be mentioned that actually some other side processes take place in the system including the process of 1,1-dinitroethane formation.

Nitrite ion produced under the experimental conditions is able to react with nitroethyl radical generating 1,1-dinitroethane [Eq. (20)]:

$$CH_3\dot{C}HNO_2 + NO_2^- \rightarrow [CH_3CH(NO_2)_2]^{\cdot-}. \quad (20)$$

Dinitroethyl radical anion may be also produced according to scheme (21):

$$CH_3CH{=}NOO^- + NO_2^{\cdot} \rightarrow [CH_3CH(NO_2)_2]^{\cdot-}. \quad (21)$$

The molecule of oxyhemoglobin may accept one electron from 1,1-dinitroethane radical anion according to Eq. (22) with a subsequent decomposition of oxyhemoglobin radical anion [Eq. (3)]. 1,1-Dinitroethane produced in reaction (22) under the experimental conditions is ionized (pK_a = 5.21 [86]) and equilibrium (23) is essentially completely shifted to the right. In this case 1,1-dinitroethane anion may be spectrophotometrically detected in the reaction medium.

$$[CH_2CH(NO_2)_2]^{\cdot-} + HbO_2 \rightarrow [HbO_2]^{\cdot-} + CH_3CH(NO_2)_2, \quad (22)$$

$$CH_3CH(NO_2)_2 \rightleftarrows CH_3C(NO_2)_2^- + H^+. \quad (23)$$

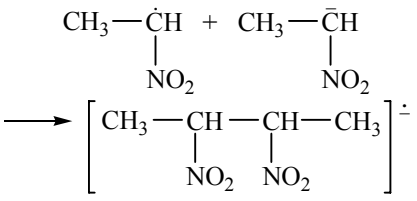
The generation of 1,1-dinitroethane under the experimental conditions is confirmed by the analytical

method which is described in detail in the experimental part. At the initial concentration of nitroethane 0.05 M, the concentration of hemoglobin 5.05×10^{-5} hem l^{-1} and pH 8.05 1,1-dinitroethane was detected in the reaction medium in the concentration 5.9×10^{-4} M. The concentration of 1,1-dinitroethane generated in this experiment amounts to about 1.2% of the initial concentration of nitroethane in the reaction medium. The percentage of the produced 1,1-dinitroethane increases with the nitroethane concentration in the reaction medium. At pH 8.0, the concentration of hemoglobin 3.05×10^{-5} hem l^{-1} and the nitroethane concentration 0.11 M after the sedimentation of hemoglobin 1,1-dinitroethane was detected in the reaction medium in the concentration 2.7×10^{-5} M which comprises 2.5% of the initial concentration of nitroethane in the system. A two-fold increase in the initial concentration of nitroethane and a two-fold decrease in the concentration of hemoglobin result in a two-fold increase in the amount of 1,1-dinitroethane produced in the system. On the basis of the obtained results it may be concluded that nitroethyl radical produced in the process simultaneously interacts with deoxyhemoglobin [Eq. (24)] and nitrite ion generated in reaction (20).

$$\mathrm{Hb(Fe^{+2})} + \mathrm{CH_3{-}\dot{C}H{-}NO_2^-} \rightarrow \mathrm{CH_3{-}CH{-}NO_2} + \mathrm{MetHb(Fe^{+3})}. \quad (24)$$

So, when the hemoglobin concentration dimimishes and the concentration of nitroethane increases, the contribution of the process of generation of 1,1-dinitroethane radical anion [Eq. (20)] rises.

The process of coupling of the nitroethyl radical with nitroethane anion present in the system in large amounts also cannot be excluded [Eq. (25)]; such coupling results in the formation of 2,3-dinitrobutane radical anion [Eq. (25)].



(25)

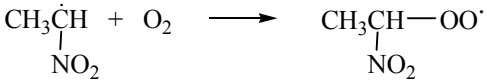
Further this species may be involved into reactions of electron transfer to the oxygenated form of hemoglobin, hydroperoxide radical or some other of the numerous electron acceptor reagents present in the system. As the result of such electron transfer 2,3-dinitrobutane is generated. This interaction cannot be ruled out because analogous reactions are widely documented in the literature [87]. The quantitative detection of the coupling products under the conditions of kinetic experiments is complicated in view of their vanishingly small concentrations. As a rule, their possible formation is just mentioned.

In our case such interaction corresponds to the removal of nitroethane radical as one of the methemoglobin producing agents from the reaction of oxidation of ferrous ion in hemoglobin and it should result in reducing the rate of methemoglobin accumulation in the system. The established correlation of the rate of methemoglobin accumulation with the concentration of nitroethane anion indirectly supports such interaction. A preliminary analysis of the data given in the table shows a weak dependence of the rate of methemoglobin production on the nitroethane concentration. But the detailed analysis of experimental results led to a rather strict correlation of the rate of methemoglobin accumulation with the concentration of molecular form of nitroethane:

$$w(\text{hem } l^{-1} \text{ min}^{-1}) = -(1.7\pm0.1)\times10^{-6}[\mathrm{CH_3CH(NO_2)}] + (2.6\pm0.2)\times10^{-7},\ r\ 0.993,\ n\ 6.$$

So the increase of nitroethane anion concentration in the reaction medium leads to the acceleration of reaction (25), more intense withdrawing of nitroethyl radical from the methemoglobin production process (24), which confirms the participation of this species in the hem oxidation process. Thus in addition to the above processes of methemoglobin generation one more independent route of hemic iron oxidation may be identified and described by the series of Eqs. (2), (5), and (24).

Taking into account that oxygen concentration in the phosphate buffer solution amounts to $(2–5)\times10^{-4}$ M [88], it may be anticipated that the nitroethyl radical generated in the course of reaction is actively transformed into nitroperoxide radical [Eq. (26)] which is able to interact with hemoglobin similarly to hydroperoxide radical [Eq. (27)]:



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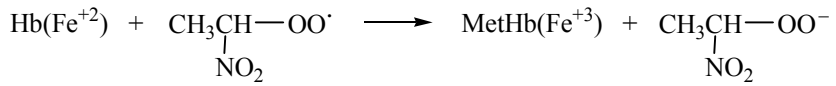
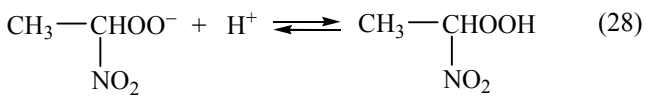


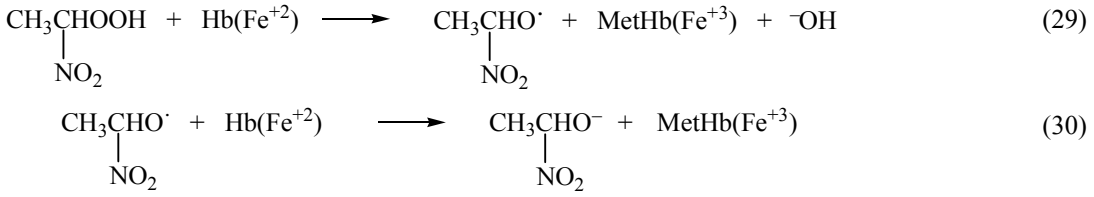
(27)

The generated nitroperoxide anion is quickly protonated in the reaction medium [Eq. (28)]



(28)

Equilibrium (28) is obviously shifted to the right taking into account the estimated value of ionization constant of the hydroperoxide of 1-chloro-1-nitroethane (p$K_a \geq 9.5$) reported in [89]. On this basis it should be anticipated that the acidity of nitroethane hydroperoxide would be much lower. So in this case the produced nitroethyl hydroperoxide would be an effective oxidizing agent for hemic iron in the deoxygenated hemoglobin molecule producing the hydroxynitroethyl radical [Eq. (29)] which is also able to oxidize hemic iron [Eq. (30)].

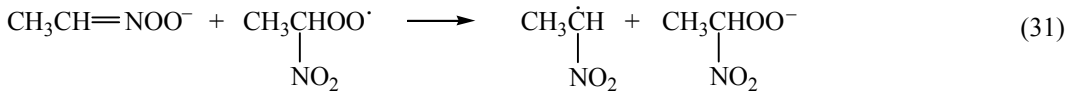


(29)

(30)

The participation of α-hydroxynitroethyl radical in this process is only hypothetical and is based on the earlier expressed suggestion about the involvement of radical $CH_3C(Cl)(NO_2)O^{\cdot}$ in the chain branching processes in ter Meer reaction [89–93] where this radical is treated as an effective electron acceptor from α-halo-α-nitroethane anion. Also in [94–97] the degeneratively branched chain process of methemoglobin generation under the action of α-halo-α-nitroalkane was also investigated in which the hydroperoxide of α-halo-α-nitroethane and α-haloxynitroethyl radical play the main roles in the reactions of chain branching. So the realization of reactions (26)–(30) is highly probable.

It should be taken in account that the anion of nitroethyl hydroperoxide may be produced with the participation of nitroethane anion [Eq. (31)] [97]:



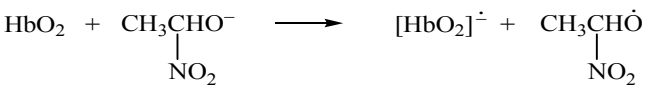
(31)

The so formed anion of nitroethyl hydroperoxide will be effectively protonated according to Eq. (28) and the resulting peroxide should readily oxidize hemic iron according to Eq. (29). The α-hydroxynitroethyl anion generated in reaction (30) may be also involved in the process producing the oxyhemoglobin radical anion according to reaction (32) analogously to that described in [94–97] during the study of the interaction of oxyhemoglobin with α-halo-α-nitroethanes. Considering a much more pronounced electron donating ability of α-hydroxynitroethyl anion in comparison with its α-halo-α-hydroxynitroethyl analogs, the unsubstituted α-hydroxynitroethyl anion is further involved into the process of methemoglobin generation [Eq. (32)]. In order to substantiate the presence of α-hydroxynitroethane anion in the reaction medium under experimental conditions, we made a theoretical estimation of its acidity.

Earlier Ballindger and Long [98] found a correlation between the acidity in an aqueous solution of substituted methanols and Taft's constants σ^*: $pK_a^{RCH_2OH} = 15.9 - 1.42\sigma_R^*$.

Assuming $\sigma^*(CH_3) = 0$ and $\sigma^*(NO_2) = 4.54$ [99], the calculated pK_a value of α-hydroxynitroethanol is estimated to be 9.45. Taking into consideration the active accumulation of nitroethyl hydroperoxide in the initial stage of the reaction, the process of methemoglobin formation through the participation of nitroethyl peroxide may be treated as a reaction initiated by this organic peroxide [Eq. (33)] where the main chain leading radical $NO_2^{\cdot}$ is generated in reaction (33) and further oxidizes hemic iron in desoxyhemoglobin [Eq. (17)].

The α-hydroxynitroethyl radical produced in reaction (33) reacts with nitrite ion according to Eq. (34)



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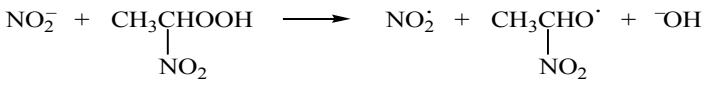
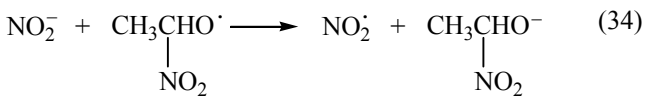


(33)

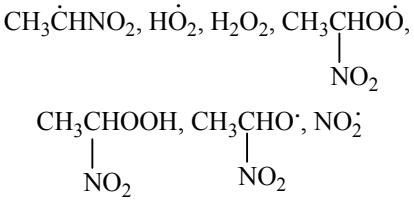
increasing the concentration of the main chain leading radical which may be treated as chain branching.



(34)

The key processes in the nitroethane-oxyhemoglobin system are interconnected through common intermediates. In the course of the reaction a lot of active species capable of oxidation of hemic iron are generated. Probably the main reactions leading to the production of species oxidizing hemoglobin to methemoglobin are the following: 2, 4, 8, 17, 20, 21, 23, 30, 32–34.

The species generated in the above reactions possessing a pronounced electron acceptor potential are listed below:



Actually in the reaction medium a complex system of interconnected chain radical processes is realized with main chain leading radicals like $HO_2^{\cdot}$, $CH_3C^{\cdot}HNO_2$, and $NO_2^{\cdot}$, whereas nitroethyl radical and nitroethane anion are the key intermediates in the generation of 1,1-dinitroethane.

EXPERIMENTAL

The investigation was carried out using hemolizates of red cells of healthy donors at (25±0.2)°C. The solutions of hemoglobin of proper concentrations were prepared in phosphate buffer solutions (pH 7.30–8.35) using the above hemolizates. Then 3 ml of the reaction solution with the help of a manual feeder was introduced into the spectrophotometer cell kept at a constant temperature for 5 min and after that the reaction was initiated by introducing 5–25 μl of nitroethane into the cell of spectrophotometer by help of a calibrated microsyringe. The optical density of the solution was measured against the initial reaction medium free of nitroethane. The color reaction with 2,4-dinitrophenylhydrazine was used to determine the amount of acetaldehyde in the system. Hemoglobin was precipitated using the traditional method [69] before measuring the amount of carbonyl compounds with the help of hydrazone-forming reaction. The precipitation was carried out after 2.5 h from the beginning of the reaction. The reaction mass was transferred to Teflon centrifuge test-tube, cooled with ice and at vigorous stirring 0.11 ml of ethanol and then 0.25 ml of chloroform per 1 ml of the reaction mass were added. The mixture was stirred on ice until hemoglobin precipitated and then the precipitate was separated by centrifugation. A colorless liquid was decanted and used for measuring the carbonyl compound concentration by means of the reaction with 2,4-dinitrophenylhydrazine. To carry out this reaction, 0.02–0.5 ml of the supernatant liquid was added to 2.5 ml of phosphate buffer (pH = 7.4) and then a solution of 2,4-dinitrophenylhydrazine ($c = 5\times10^{-4}$ M) in 0.5 M hydrochloric acid was added. After 20 min of exposition the optical density of the solution was measured at 363 nm against a buffer solution containing the same amount of 2,4-dinitrophenylhydrazine as the reference material. The concentration of the carbonyl compound was calculated on the basis of its molar extinction coefficient at 363 nm [100].

To measure dinitroethane concentration in the reaction solution, hemoglobin was also precipitated using the above method. After hemoglobin precipitation was over, the optical density of the supernatant liquid at 410 nm was measured against the buffer solution. The concentration of 1,1-dinitroethane was calculated using the extinction coefficient of 1,1-dinitroethane anion, $\varepsilon = 4.7\times10^3$ l mol^{-1} cm^{-1} [89].

CONCLUSIONS

(1) The process of methemoglobin formation under the action of nitroethane was investigated.

(2) It was shown that this process followed the chain radical mechanism and was accompanied by a lot of side reactions including the oxidative denitration of nitroethane and the formation of 1,1-dinitroethane due to involving into the process the nitrite ion generated in the reaction of nitroethane oxidative denitration.

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