CHEMILUMINESCENT RESEARCH ON THE REACTIONS BETWEEN FERRITIN, ALBUMIN AND REACTIVE OXYGEN SPECIES

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Лилия Владимирова, Елица Павлова, ХЕМИЛУМИНЕСЦЕНТНО ИЗСЛЕДВАНЕ НА РЕАКЦИИТЕ МЕЖДУ ФЕРИТИН, АЛБУМИН И АКТИВНИ ФОРМИ НА КИСЛОРОДА

С метода на луцигенин-активирана хемилуминесценция е изследвана реакцията на феритин и албумин с активни форми на кислорода. Реакциите са изучени в различни системи за генерация на свободни радикали и активни форми на кислорода (АФК): ензимна (ксантин-ксантиноксидазна) и химична (редуциран α-никотинамид-аденин динуклеотид-феназинметасулфат) системи за генерация на супероксидни радикали ($O_2^{\dot{-}}$), а така също и в система с реактив на Фентън (H_2O_2-$FeSO_4$) – за генерация на хидроксилни радикали (·OH). Изследвано е също и действието на хелатиращи вещества (десферал, аденозинтрифосфат и етилен-диамин-тетраоцетна киселина) с потискащ ефект върху интензивността на хемилуминесценция в тези окислително-редукционни процеси. Изчислени са константите на инхибиране за феритин и албумин и вещества с доказана хелаторна активност. Показана е разликата в техните ефективности в зависимост от стойностите на pH и типа на съответната система за генерация на свободни радикали и АФК.

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We investigated the interactions of ferritin and albumin with reactive oxygen species (ROS) by the lucigenin-enhanced chemiluminescence method. These reactions are explored in different systems for generation of ROS: enzymatic (xanthine – xanthineoxidase) and chemical (phenazinemethosulfat-reduced α-nicotinamide adenine dinucleotide) – for the generation of $O_2^{\dot{-}}$-radicals, and Fenton's system (H_2O_2 – Fe^{2+}) – for the generation of ·OH- radicals. We also studied the effects of three chelators (desferal, ethylenediamineteraacetic acid and adenos-

ine triphosphate) suppressing these redox processes. Their activity corresponds to different intensity of chemiluminescence. The constants of inhibition about ferritin, albumin and these substances with proven chelative effects are calculated. Their effect varies depending on pH and the type of the model system applied for the generation of ROS.

Keywords: chemiluminescence; ferritin; albumin; chelators; reactive oxygen species.
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1. INTRODUCTION

It is well established that anemia is often accompanied with chronic hypoxia and high concentrations of serum iron. Iron overload switches various pathological processes on, including oxidative damage to the erythrocyte membranes as well as other membrane structures [1]. These processes are accompanied with metabolic overproduction of free radicals and reactive oxygen species (ROS), activation of the free radical lipid peroxidation cascade and production of toxic metabolites [2]. The accumulation of free radical intermediates overwhelms the antioxidant capacity of the organism (content of endogen antioxidants and antioxidants enzymes). Oxidative stress appears one of the basic mechanisms in the progression of diseases caused by high content of active iron [3, 4].

Albumin is one of the proteins with highest plasma concentrations (about 42 g/l), with various structure and transport functions. Its high concentration defines over 80% of the blood plasma oncotic pressure; the 18 negative charges of this molecule enable one of its major functions – balance of the ion content of all tissue liquids. Albumin is also a metabolic transporter of free fatty acids, hormones, fat-soluble vitamins, different drugs as well as great quantities of Cu^{2+}. It is a chelative agent to ions with changing valency, such as Cu^{2+} [5].

Ferritin is the reservoir of iron in the organism [6]. Probably, the Fe^{2+}-ions are transported through the protein channels inside the molecule, where they are oxidized and kept as FeOOH-nucleus in combination with non-stechiometric quantities of phosphates [7].

Many authors have researched the role of oxygen radicals in the biochemical interactions of ferritin [8, 9,10]. The superoxide radicals ($O_2^{\dot{-}}$) affect the Fe^{3+}-mobilization from ferritin [11, 12, 13]. On the other hand, higher concentrations of ferric iron cause activation of the free radical lipid peroxidation process [14].

For the proposed reasons it is important to research the opportunities of effective therapy on patients with iron and other metals overload by different chelative agents of the changing valency ions.

The aim of this experimental work is to study the interactions of ferritin and albumin with ROS ($O_2^{\dot{-}}$, ·OH) by the method of lucigenin-enhanced chemiluminescence (CL). The applied model systems are chemical and enzymatic – Fenton's system, reduced α-nicotinamide adenine dinucleotide – phenazinemethosulfate and xanthine–xanthineoxidase. There are calculated the constants of inhibition about these proteins and substances with proven chelative activity (desferal, ethylenediamineteraacetic acid (EDTA) and adenosine triphosphate (ATP)).

2. MATERIALS AND METHODS

1. *Chemiluminescent systems*: Each sample contains: Fenton`s reagent ($FeSO_4$ (5.10^{-5} M) – H_2O_2 (5.10^{-4} M)), xanthine (0.33 mM) – xanthineoxidase (0.3 IU) or NAD.H (10^{-4} M) – PMS (10^{-6} M). The chemiluminescent probe lucigenin (10^{-4} M) as well as the tested substance (albumin 0.01, 0.04 mg/ml; ferritin 0.01 mg/ml; desferal 10^{-4} M, ATP 10^{-4} M, EDTA 10^{-4} M) is added to each sample. All the three systems are tested at pH 6.0, pH 7.4 and pH 8.0 / 25°C.

The following buffer solutions are used: Na_2HPO_4 (0.05 M) – $C_8H_8O_7.H_2O$ (0.1 M), at pH 6.0, 7.4 and 8.0 / 25°C.

2. *Reagents*: horse spleen ferritin, Type I: from Horse Spleen; sterile filtered solution in 0,5 M NaCl (Sigma Chemical Co. USA); human serum albumin (Reanal, Hungary); iron sulphate $FeSO_4$, ethylene diamine tetracetic acid $C_{10}H_{16}N_2O_8$ (EDTA), phenazine methosulfate $C_{13}H_{11}N_2.CH_3SO_4$ (PMS) (N-methyldibenzopyrazine methyl sulfat salt), xantine $C_5H_4N_4O_2$ (2,6-dihydroxypurine), xantine oxidase (Merck KGaA, Germany); adenosine triphosphate $C_{10}H_{16}N_5O_{13}P_3$ (ATP) (Serva); Deferoxamine $C_{25}H_{48}N_6O_8$ (desferal) (Novartis, Switzerland); hydrogen peroxide H_2O_2, disodium hydrogen phosphate Na_2HPO_4, citric acid $C_8H_8O_7.H_2O$ (Boron, Bulgaria); lucigenin $C_{28}H_{22}N_4O_6$ (bis-N-methylacridinium nitrate), α – nicotinamide adenine dinucleotide, reduced form $C_{21}H_{27}N_7O_{14}P_2Na_2$ (NAD.H) (Boehringer, Germany).

The given concentrations are the ones in the tested mixture, as the total volume of each sample is 2 ml. The kinetics is registered for 3 minutes period. All the measurements are made with chemiluminometer LKB 1251 (Sweden) in new plastic cuvettes.

3. RESULTS

The integral evaluation of the content of free radicals and ROS as well as the registration of the kinetic parameters of the oxidation reaction could be easily made by the CL-method.

The constant of inhibition can be calculated according to the CL kinetics by the equation [15]:

$$[(dI/I_0)dt]_{t=0} = -2k[InH]_0 \quad (1)$$

where: $[[(dI/I_0)dt]_{t=0}$ is tgα (the tangent of the angle between the kinetic straight line and the ordinate) in the first period of fast CL emission; $[InH]_0$ is the concentration of the inhibitor.

The achieved results show that highest CL signal is emitted from the Fenton's system, in comparison to the other two tested systems. The intensity of oxidation is very intensive in the first seconds of the reaction for generation of hydroxyl radicals (·OH). The CL signal is strong and stable during the registered Fenton's reaction (Fig.1a).

The oxidative burst caused by the generation of $O_2^{\dot{-}}$-radicals in the NAD.H – phenazine metosulfate system is observed in the first 20 seconds from the start of the reaction; afterwards the CL response is quickly decreasing (Fig.1b).

The peak for generation of $O_2^{\dot{-}}$-radicals is detected about the 20^{-th} second in the 3rd system: xanthine–xanthine oxidase; the CL response is gradually decreasing with time (Fig.1c).

One should take in mind that the intensity of the CL signal depends proportionally on the alkality of the medium in all three tested systems.

We should also notice that the registration of CL emitted from NAD.H – phenazine metosulfate and xanthine–xanthine oxidase systems is possible with the application of a specific $O_2^{\dot{-}}$ -radicals probe–lucigenin (Fig.1 b), c)) [16].

a) b)

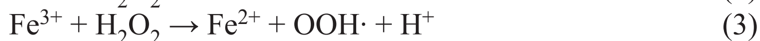
c)

Fig.1. Intensity of chemiluminescence and kinetics of the control systems at pH 6.0/25oC, pH 7.4/25oC, pH 8.0/25oC: a) Fenton`s system (FeSO4-H2O2) b) NAD.H – phenazine metosulfate c) xanthine –xanthine oxidase

A. FENTON'S SYSTEM

It is well known that the interaction between Fe^{2+}-ions and H_2O_2 (Fenton's reaction) results in highly reactive, short-living ·OH-radicals attacking free fatty acids and proteins. The CL emission from this reaction is much higher in comparison to the other two systems:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH^- \quad (2)$$



$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OOH\cdot + H^+ \quad (3)$$

The release of Fe^{3+}-ions from the ferritin nucleus initiates a second step interaction with hydrogen peroxide in the tested *in vitro* system, which is accompanied with large increase of the registered CL signal. On the contrary, the inclusion of Fe^{3+}-ions inside the ferritin nucleus decreases the CL response.

The registered high CL signal from the samples containing albumin and Fenton's reagent could be explained with oxidation of the molecule according to the standard scheme of initiation of radical protein peroxidation.

The achieved results confirm that the oxidation reaction is stimulated at pH 8.0 and the following protein content–ferritin (0.01 mg/ml), albumin (0.04 mg/ml) (Table 1). Ferritin easily participates in free radical oxidation processes at physiological conditions pH (7.4/25°C); albumin (0.01 mg/ml) demonstrates chelative effect at acid, neutral and alkal pH when applied in concentration 4-times lower than the average physiological content (Table 1). Should be noticed that albumin (0.04 mg/ml) promotes oxidation described with smooth kinetics for the tested range of pH (Table 1).

The experimental data show that ferritin possesses no antioxidant activity towards the generated ·OH-radicals in the Fenton's system. This fact can be explained with the oxidation of the protein partition of the molecule as well as the mobilization of Fe^{3+}-ions outside the ferric nucleus.

The addition of desferal in equal ratio to the content of Fe^{2+}-ions in the Fenton's system strongly inhibits the CL emission at pH 6.0 and pH 8.0 and slightly at pH 7.4. This could be explained with the active mechanism of desferal:

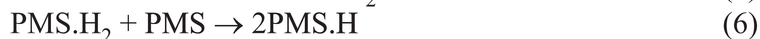
$$Fe^{2+} + \text{desferal} + O_2 \rightarrow Fe^{3+}\text{-desferal} + O_2^{\dot{-}} \quad (4)$$

When ATP and EDTA are applied in the same dose (10^{-4}M), they exhibit strong chelative effect at pH 8.0/25 °C in Fenton's system, which does not present at pH 7.4/25 °C (Table 1).

B. NAD.H – PHENAZINE METOSULFATE SYSTEM

Fast oxidation and generation of $O_2^{\dot{-}}$-radicals is observed in the first stage of spontaneous CL from the NAD.H–PMS system [17]:

$$NAD.H + PMS + H^+ \rightarrow PMS.H_2 + NAD^+ \quad (5)$$



$$PMS.H_2 + PMS \rightarrow 2PMS.H \quad (6)$$

$$PMS.H^{\cdot} + O_2 \rightarrow PMS + O_2^{-\cdot} + H^+ \quad (7).$$

The antioxidant effect of ferritin is observed precisely at this phase of the

Table 1. Intensity of lucigenin-enhanced chemiluminescence at pH 6.0, pH 7.4, pH 8.0 / 25 °C in Fenton`s system: ferritin ($I_{ferr} - I_c$), albumin (0.04 mg/ml) ($I_{alb1} - I_c$), albumin (0.01 mg/ml) ($I_{alb2} - I_c$), desferal ($I_{desf} - I_c$), ATP ($I_{ATp} - I_c$), EDTA ($I_{ATp} - I_c$)

	pH 6.0						pH 7.4						pH 8.0					
time	$I_{ferr} - I_c$	$I_{alb1} - I_c$	$I_{alb2} - I_c$	$I_{desf} - I_c$	$I_{ATP} - I_c$	$I_{EDTA} - I_c$	$I_{ferr} - I_c$	$I_{alb1} - I_c$	$I_{alb2} - I_c$	$I_{desf} - I_c$	$I_{ATP} - I_c$	$I_{EDTA} - I_c$	$I_{ferr} - I_c$	$I_{alb1} - I_c$	$I_{alb2} - I_c$	$I_{desf} - I_c$	$I_{ATP} - I_c$	$I_{EDTA} - I_c$
s	mV	mV	mV	mV	mV	mV	mV	mV	mV	mV	mV	mV	mV	mV	mV	mV	mV	mV
0	0.33	0.35	-0.26	-0.37	-0.23	0.12	5.15	4.77	-2.02	-0.68	0.51	2.31	12.43	19.89	-7.28	-5.83	-1.80	-7.80
3	0.28	0.37	-0.24	-0.35	-0.19	0.13	5.45	5.72	-1.19	0.18	1.44	3.14	11.51	19.31	-6.99	-6.05	-1.36	-7.59
6	0.27	0.30	-0.24	-0.32	-0.18	0.14	5.42	5.80	-1.15	0.14	1.32	3.14	11.39	19.07	-6.84	-6.23	-1.80	-7.43
9	0.28	0.33	-0.22	-0.29	-0.18	0.15	5.39	6.02	-0.95	0.35	1.56	3.29	10.84	19.23	-7.01	-6.40	-1.97	-7.61
12	0.29	0.31	-0.20	-0.28	-0.16	0.14	5.46	6.07	-0.91	0.37	1.57	3.27	10.98	18.41	-6.96	-6.49	-1.86	-7.37
15	0.27	0.31	-0.21	-0.26	-0.12	0.14	5.41	6.11	-0.84	0.37	1.54	3.27	10.56	18.34	-6.78	-6.59	-1.68	-7.30

oxidation kinetics for all tested pH. Albumin inhibits the oxidation at pH 6.0 in both applied concentrations; at pH 7.4 is effective only its lower concentration; at pH 8.0 neither albumin, nor ATP and desferal exhibit suppressive effect towards the free radical process (Fig. 2).

Desferal, ATP and EDTA inhibit the oxidation reaction and the generation of superoxide radicals at pH 6.0 and pH 7.4. Most effective is EDTA, which suppresses the oxidation reaction at all tested pH of the medium (Fig. 2).

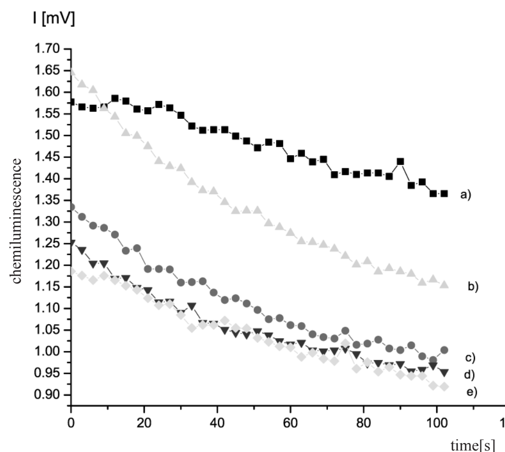


Fig.2. Intensity of lucigenin-enhanced chemiluminescence:
a) NAD.H 10^{-4} M + PMS 10^{-6} M + lucigenin 10^{-4} M + buffer pH 7.4/25 °C (control);
b) NAD.H 10^{-4} M + PMS 10^{-6} M + lucigenin 10^{-4}M + buffer pH 7.4/25 °C + albumin 0.04 mg/ml;
c) NAD.H 10^{-4} M + PMS 10^{-6} M + lucigenin 10^{-4} M + buffer pH 7.4/25 °C + ferritin 0.01 mg/ml;
d) NAD.H 10^{-4} M + PMS 10^{-6} M + lucigenin 10^{-4} M + buffer pH 7.4/25 °C +

C. XANTHINE – XANTHINE OXIDASE SYSTEM

The level of enzyme oxidation in the reaction

$$C_5H_4N_4O_2 \xrightarrow[O_2 + H_2O]{\text{xanthine oxidase}} C_5H_4N_4O_3 + H_2O_2 \qquad (8)$$

is proportional to the generation of $O_2^{\dot{-}}$-radicals; products of this interaction are uric acid and hydrogen peroxide.

Active generation of $O_2^{\dot{-}}$ -radicals in this system is registered at the 2nd stage of CL – fast flash (Fig. 3). The researched protein molecules and chelative agents inhibit the oxidation process at pH 6.0 and pH7.4. Ferritin and albumin show prooxidant activity at pH 8.0, which is interpreted with oxidation

of the protein partitions of these molecules (Fig.3). Therefore chelative agents enable the release of active iron from ferritin [17]. Most effective chelator in this enzyme system is EDTA, at alkal pH (Fig. 3).

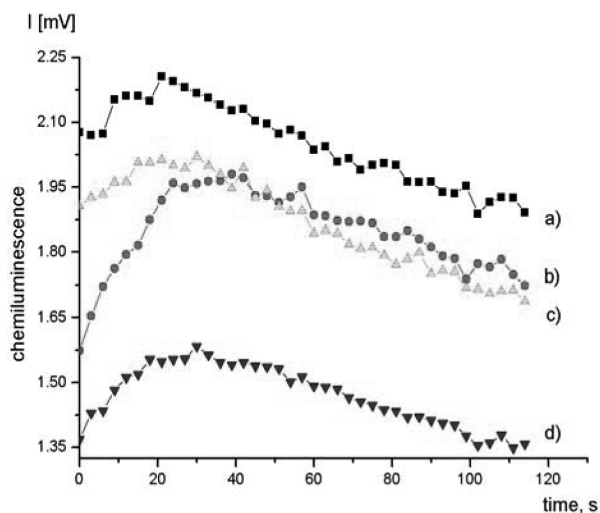


Fig. 3. Intensity of lucigenin-enhanced chemiluminescence:
a) xanthine 0.33 mM + xanthine oxidase 0.3 IU + lucigenin 10^{-4} M + buffer pH 7.4/25 °C (control); b) xanthine 0.33 mM + xanthine oxidase 0.3 IU + lucigenin 10^{-4}M + buffer pH 7.4/25 °C + albumin (0.04 mg/ml); c) xanthine 0.33 mM + xanthine oxidase 0.3 IU + lucigenin 10^{-4} M + buffer pH 7.4/25 °C + albumin (0.01 mg/ml); d) xanthine 0.33 mM + xanthine oxidase 0.3 IU + lucigenin 10^{-4} M + buffer pH 7.4/25 °C + ferritin (0.01 mg/ml)

D. CALCULATION OF THE CONSTANTS OF INHIBITION

The constants of inhibition about the tested proteins (albumin and ferritin) and chelators (desferal, ATP and EDTA) are calculated. Albumin appears most effective inhibitor of the free radical oxidation in all three examined systems, when applied in concentrations 4-times lower than physiological health levels (Table 2).

Desferal, EDTA and ATP possess strongest chelative activity in Fenton's system at alkal pH. These facts are easily explained with the high content of active iron (Fe^{2+}) in the system for generation of $^{\cdot}OH$-radicals in comparison to the other two systems (for generation of $O_2^{\cdot-}$-radicals). Lowest values of the constants of inhibition are calculated for these substances in the xanthine–xanthine oxidase system at pH 6.0 (Table 3).

Table. 2. Constants of inhibition in Fenton`s ($FeSO_4$-H_2O_2), NAD.H - phenazine metosulfate and xanthine – xanthine oxidase systems at pH 6.0, pH 7.4, pH 8.0 / 25 °C: albumin (0.01 mg/ml; 0.04 mg/ml), ferritin (0.01 mg/ml)

System	Fenton's			NAD.H – phenazine metosulfate			Xanthine – xanthine oxidase		
	K ($mg/ml.sec^{-1}$) pH 6.0	*K* ($mg/ml.sec^{-1}$) pH 7.4	*K* ($mg/ml.sec^{-1}$) pH 8.0	K ($mg/ml.sec^{-1}$) pH 6.0	*K* ($mg/ml.sec^{-1}$) pH 7.4	*K* ($mg/ml.sec^{-1}$) pH 8.0	K ($mg/ml.sec^{-1}$) pH 6.0	*K* ($mg/ml.sec^{-1}$) pH 7.4	*K* ($mg/ml.sec^{-1}$) pH 8.0
Albumin 0.04 mg/ml	0.36	1.08	3.55	0.27	0.40	3.55	-0.05	0.25	1.88
Albumin0.01 mg/ml	0.19	0.09	2.17	0.18	0.34	0.81	0.01	0.17	2.89
Ferritin 0.01 mg/ml	0.75	4.27	9.36	0.26	0.26	0.55	0.03	0.12	0.49

Table. 3 Constants of inhibition in Fenton`s ($FeSO_4$-H_2O_2), NAD.H - phenazine metosulfate and xanthine – xanthine oxidase systems at pH 6.0, pH 7.4, pH 8.0 / 25 °C: ATP (10^{-4}M), EDTA (10^{-4}M), desferal (10^{-4}M)

System	Fenton's			NAD.H – phenazine metosulfate			Xanthine – xanthine oxidase		
	K ($M.sec^{-1}$) pH 6.0	*K* ($M.sec^{-1}$) pH 7.4	K ($M.sec^{-1}$) pH 8.0	K ($M.sec^{-1}$) pH 6.0	*K* ($M.sec^{-1}$) pH 7.4	K ($M.sec^{-1}$) pH 8.0	K ($M.sec^{-1}$) pH 6.0	K ($M.sec^{-1}$) pH 7.4	K ($M.sec^{-1}$) pH 8.0
ATP 10^{-4} M	36.15	175.45	545.4	4.94	15.35	45.15	- 1.96	16.95	22.2
EDTA 10^{-4}M	48.55	111.65	653.5	16.8	7.1	120.7	1.06	2.15	7.5
Desferal 10^{-4}M	23.35	139.35	714.5	29.45	22.2	81.5	5.2	6.5	47.95

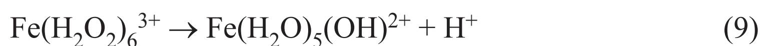
4. DISCUSSION

Three stages are defined in the kinetics of the reaction between ferritin, albumin and ROS: spontaneous (0–20 s), fast flash (20–40 s) and latent period (50–200 s). The interaction between ferritin, albumin and free radicals and ROS, generated in the system, decreases sharply the CL-response. The calculated constants of inhibition demonstrate that albumin possesses strong antioxidant activity when applied in lower than physiological values; ferritin is most effective in the NAD.H–PMS system, for the whole range of tested pH levels.

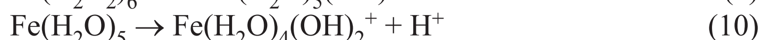
The complex-forming agents (desferal, ATP, EDTA) are able to connect tightly with the iron ions. It should be noticed that Fe^{3+}-salts which are formed in the organism are not stable. They exist *in vivo* mainly as complex substances with adenylphosphates and organic acids. Thus no quantities of the insoluble $Fe(OH)_3$ are formed [1].

There exist stable compounds with iron in the organism, which appear its transporters. For instance, deferoxamine (desferal) is applied in medicine as a chelator to active free iron ions in patients with β-thalassemia major [18].

Desferal activates the auto-oxidation of ferric ions. This is confirmed with lower Fe^{2+}-concentrations and higher Fe^{3+}- and oxygen radicals` concentrations. Protons are detached in these reactions. The mechanism is through step-by-step hydrolysis:



$$Fe(H_2O_2)_6{}^{3+} \rightarrow Fe(H_2O)_5(OH)^{2+} + H^+ \quad (9)$$

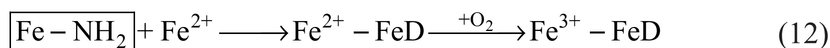


$$Fe(H_2O)_5 \rightarrow Fe(H_2O)_4(OH)_2{}^{+} + H^+ \quad (10)$$

$$Fe(H_2O_2)_5(OH)^{2+} \rightarrow Fe(H_2O)_8(OH)_2{}^{2+} + 2H_2O \quad (11)$$

As a result, desferal removes the water molecules from the six coordinative locations of Fe^{3+}, suppresses its hydrolysis forming iron complexes. Desferal binds Fe^{3+} by the $–NH_2$ groups releasing 3 proton particles [19].

Probably, the iron release from the ferritin molecule is according to the following scheme:



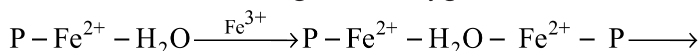
$$\boxed{Fe - NH_2} + Fe^{2+} \longrightarrow Fe^{2+} - FeD \xrightarrow{+O_2} Fe^{3+} - FeD \quad (12)$$

$$FeD \longrightarrow Fe^{2+} \quad (13)$$

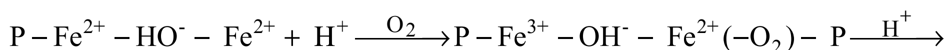
$$F \longrightarrow Fe^{2+} \longrightarrow FRox. \quad (14)$$

Two Fe^{2+}-atoms are bound to the protein (P) in close proximity, allowing the formation of an ^{-}OH-bridged Fe^{2+} dimmer. The oxygen molecule binds to

one of the Fa^{2+}-atoms allowing oxidation of both of them with concomitant formation of H_2O_2 [20]. The oxidation of the two Fe^{2+}-ions occurs without prior formation of an ^{-}OH-bridge between them, but with bridging either through a water molecule or through the dioxygen itself.



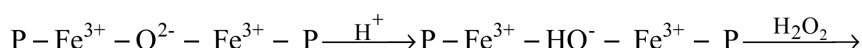
$$P - Fe^{2+} - H_2O \xrightarrow{Fe^{3+}} P - Fe^{2+} - H_2O - Fe^{2+} - P \longrightarrow$$



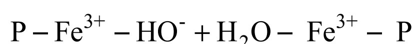
$$P - Fe^{2+} - HO^{-} - Fe^{2+} + H^{+} \xrightarrow{O_2} P - Fe^{3+} - OH^{-} - Fe^{2+}(-O_2) - P \xrightarrow{H^{+}}$$

$$P - Fe^{3+} - O^{2-} - Fe^{3+} - P + H_2O_2.$$

It is established that after its oxidation, the ferritin molecule is split to numerous dimmer structures, which leads to migration of one or two atoms from the ferroxidase center of the iron nucleus through the three-fold channels:



$$P - Fe^{3+} - O^{2-} - Fe^{3+} - P \xrightarrow{H^{+}} P - Fe^{3+} - HO^{-} - Fe^{3+} - P \xrightarrow{H_2O_2}$$



$$P - Fe^{3+} - HO^{-} + H_2O - Fe^{3+} - P$$

One electron oxidation is possible only if one Fe^{3+}-ion is left in the ferroxidase center and is reduced to Fe^{2+}.

There exists an earlier oxidation scheme which differs from the previous one. According to it, the oxygen molecule is connected between two Fe^{2+}-atoms of the adjacent subunits and both oxygen atoms are joined later to the nucleus [21].

Free ferric ions activate the processes of free radical oxidation and generation of ROS. These reactions could be catalyzed by other metals with changing valency too. But only ferric ions are in sufficient concentrations *in vivo*. Erythrocytes injury and the release of active iron in the serum cause serious cell damage after its reaction to oxygen and the subsequent generation of cytotoxic radical intermediates [22]. The compensation therapy of oxidative stress in such pathology is by chelators, such as desferal, which control and prevent the accumulation of free active iron within the tissues, suppress the generation of ROS and regulate the process of free radical lipid peroxidation [23]. These facts show the actuality of one of the proposed tasks of this experimental research – comparison between the chelative effects of desferal, ATP and EDTA in different systems for generation of free radicals and ROS, in a range of acid, neutral and alkal pH of the medium.

One should take into advance that chelators change the potentials of oxidation and reduction about the Fe^{3+}/ Fe^{2+} pair in water solution. It is 0.77 V about the free ions. The redox potential of the Fe^{3+}/ Fe^{2+} pair is decreased in the reaction with complex-forming agents, which facilitates the electron trans-

fer from Fe^{2+} to other structures, including dioxygen (O_2) (Table 4). A simple example about that fact is the auto-oxidation of Fe^{2+} in water solutions with different alkality. $Fe(OH)^+$ and $Fe(OH)^{2+}$ are produced if hydroxide ions (^-OH) are present within the medium. The redox potential of the $Fe(OH)^+$/ $Fe(OH)^{2+}$ pair is 0.30 V. This results in fast iron oxidation in alkal medium (the redox potential of the $O_2^{-\cdot}/O_2$ pair is –0.33 V). The irreversibility of this reaction is secured by the production of $Fe(OH)_3$ sediment. On the contrary, the solutions of Fe^{2+} in acid medium are stable because the production of $Fe(OH)_3$ is not possible [1]. Fast oxidation of Fe^{2+} can also be observed in the presence of EDTA, orthophosphate, desferal and citrate. These reagents cause decrease on the redox potential of the Fe^{3+}/ Fe^{2+} pair (Table 4).

Table 4. Redox potential values about the Fe^{3+}/ Fe^{2+} pair, measured in the presence of complex-forming agents at pH 7.0

Reaction	$E^{0'}$ (V)
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	0.77
$Fe(OH)^{2+} + e^- \rightarrow Fe(OH)^+$	0.33
EDTA-Fe^{3+} + e^- → EDTA-Fe^{2+}	0.12
Desferal-Fe^{3+} + e^- → desferal-Fe^{2+}	–0.45
1,10 phenatropin-Fe^{3+} + e^- → 1,10 phenatropin-Fe^{2+}	1.1
Cytochrome c-Fe^{3+} + e^- → cytochrome c-Fe^{2+}	0.27
Ferritin-Fe^{3+} + e^- → ferritin-Fe^{2+}	–0.19
Transferrin-Fe^{3+} + e^- → transferrin-Fe^{2+}	0.40

Some authors prove that the mixture of ferric ions and desferal results only in the generation of hydroxyl radicals (·OH) [22]. The experiment is performed in acid medium, where most of the products are hydroperoxide radicals ($HO_2\cdot$) – a result from the oxidation of iron. It dismutates as the product of this reaction is hydrogen peroxide (H_2O_2) which is the source of hydroxyl radicals (·OH). Other authors register the production of superoxide radicals ($O_2^{\dot{-}}$) within the same system [22, 23]. The experiment is performed at pH 7.4. Within these conditions predominantly are formed long-living superoxide radicals ($O_2^{\dot{-}}$) in advantage to the short-living hydroxyl radicals (·OH). These data support the thesis that the complex-forming agents change the redox potential of the Fe^{3+}/ Fe^{2+} pair, accelerate the oxidation of iron, accompanied with the generation of ROS. Hence the reagents forming complexes with iron could possess prooxidant or antioxidant activity depending on the conditions of the reaction. The antioxidant activity is defined with the fast oxi-

dation reaction between Fe^{2+}-ions and chelators which switches them off other possible oxidation. The prooxidant activity depends on the oxidation reaction between Fe^{2+} and oxygen and the subsequent generation of ROS. One should also take in mind that some complex-forming agents maintain Fe^{3+}-ions in soluble, catalyc active state.

Finally, we can define three different mechanisms of the activity of the complex-forming agents towards ferric ions. The first option is to extract these ions from the redox reactions. The second effect is the possibility to produce complex compounds, which do not hinder the participation of ferric ions in redox reactions but counteract the sedimentation of insoluble iron hydroxides. The third alternative is to change the redox potential of ferric ions with subsequent acceleration or detention of the redox reactions.

5. CONCLUSION

The chemiluminescent method is suitable to research the interactions between ferritin, albumin and ROS in model systems by.

The achieved results demonstrate that ferritin possesses no antioxidant activity in the Fenton's system which is probably due to oxidation of the protein partition of the molecule. It shows the following effects in the other two tested systems: NAD.H-phenazine metosulfate – antioxidant activity about the whole range of tested pH; xanthine–xanthine oxidase – prooxidant effect.

Albumin stimulates smoothly the oxidation reaction when applied in physiological concentrations in the Fenton's system at all tested pH of the medium. Its chelative effect is manifested when applied in lower concentration at alkal, neutral and acid pH. Albumin exhibits no effect of inhibition in the systems NAD.H-phenazine metosulfate and xanthine–xanthine oxidase at pH 8.0.

The experimental data confirm that desferal, ATP and EDTA are most effective chelators in the Fenton's system about the whole range of tested acidity which is linked to the alternations of the redox status of ferric ions.

The combined therapy with effective chelators and natural or synthetic antioxidants could be very helpful in clinical practice as far as the oxidative stress condition in pathology is accompanied with high concentrations of free radicals and ROS, accumulation of free radical lipid peroxidation products and suppressed antioxidant capacity in the organism.

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