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THE STUDY OF THE KINETIC AND THERMODYNAMIC OF THE BLOOD SERUM PROPERTIES FREE- RADICAL OXIDATION LIPIDS APPLYING THE METHOD OF CHEMILUMINESCENCE

Keywords: chemiluminescense, blood serum, energy of activation, lifetime

We investigated the physical parameters of chemical reactions which take place in blood serum (BS) at the chemiluminescence (CL) accompanying the process of peroxide oxidization of lipids. The emission intensity, wavelength, lifetime and energy of activation of the CL BS are sensitive to the pathologies. Under investigation CL spectrum it was found essential distinctions in the luminescence intensity in the presence of pathological processes such as tuberculosis and cancer. It was established that the ketonic compounds luminescence. Using a Stern-Volmer the lifetime of excited states equation was found. The data obtained suggest that ketones are in triplet states make the main contribution into CL BS.. An empirical Arrhenius equation for FRO was offered. Dependences of total chemilum inescence on the temperature were estimated. The energy of activation was determined differences of pathological processes were found. It is shown that this parameters change under the condition of pathological processes such as tuberculosis and cancer.

1. INTRODUCTION

The problem of free- radical oxidation (FRO) of lipids is one of the central problems in modern molecular biophysics [1,3]. It is established, that FRO proceeds persistently in all tissues of the living organism, and free- radical processes at low intensity serve as one of the typical metabolic processes. Both augmentation and delay of FRO in biological object [5] lead to various pathologies. The most complete information concerning kinetic and thermodynamic properties of FRO can be obtained experimentally by means of the method of chemiluminescence (CL); this motivates the elaboration of systems connected with this method of investigation.

Interaction- recombination between peroxides radicals cause the CL, which in its can initiate the photochemical processes of electron excitation and formation of radicals. FRO of carbohydrates and their derivatives is the chain radical reaction. The reaction is lead by carbohydrate $R^{\bullet}$ and peroxide radical $RO^{\bullet}$. The mechanism of the first stage, namely the formation of the hydroperoxide $ROOH$, consist of the following elementary processes:

$$\text{Initiating of the cain with rate } w_i \qquad (1)$$

$$\left.\begin{aligned} R^{\bullet} + O_2 &\xrightarrow{k_2} RO_2^{\bullet} \\ RO_2^{\bullet} + RH &\xrightarrow{k_3} R^{\bullet} + ROOH \end{aligned}\right\} cain \qquad (2,3)$$

$$R^{\bullet} + R^{\bullet} \xrightarrow{k_4} R_2 + h\gamma \qquad (4)$$

$$R^{\bullet} + RO_2^{\bullet} \xrightarrow{k_5} ROOH + h\gamma \qquad (5)$$

$$RO_2^{\bullet} + RO_2^{\bullet} \xrightarrow{k_6} spirit + ketone + O_2 + h\gamma \qquad (6)$$

Excited luminescence takes place in (4), (5), (6) exothermal processes due to the energy, that release at recombination (or disproportionation) of radicals; correspondingly the initiated luminescence intensity is proportional to the rate of the radical recombination. In the stable regime, the intensity of CL is proportional to the chain initiation rate.

The formation of O_2 in biological systems is accompanied by the H_2O_2 formation as a result of the dismutation of superoxidedismutase (SOD):

$$2O_2^{\bullet -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \qquad (7)$$

2. DESCRIPTION OF THE EXPERIMENTAL SETUP

The experiments were carried out on modified biochemical analyzer which has the following technical parameters:

- sensitivity of 10^4 quanta/sec,
- working sample volume – not greater than 1ml,
- electronic accuracy temperature control $1^{\circ}C$,
- temperature diapason has been changed from $20^{\circ}C$ to $45^{\circ}C$,
- spectral range 300 – 750 nm.

The scheme of the setup is shown in Fig. 1

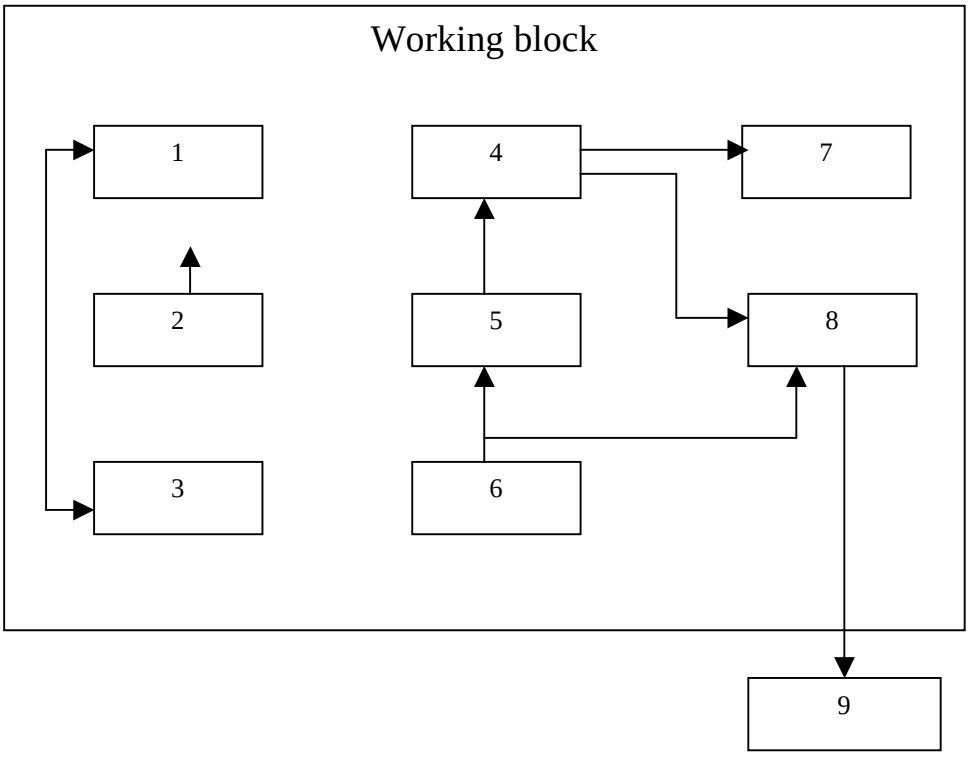

Fig. 1. Schematic of the biochemical analyzer; 1 – switch key, 2 – termostabilization board, 3 – mixing regulation, 4 – electron photomultiplier, 5 – power supply, 6 – power supply stabilization, 7 – counting amplifier, 8 – analog amplifier, 9 – analog-digital converter

3. EXPERIMENTAL RESULTS

Our study aims to investigate the physical parameters of chemical reactions. Taking place in BS at the CL, in particular, to determine, which of the three later reactions contributes most to the process. The aim of the investigation was also to determine the changes of these parameters under the condition of mutually antithetic pathological processes as tuberculosis and cancer, and comparing this change with the norm.

Under investigation of CL spectrum of 60 samples BS people (20 samples BS blood-donors, 20 samples BS tubercular patients and 20 samples-cancerous patients) it was fixed that, the peaks are in the region of 500 nm was found, the halfwidth of the zone equals to 150 nm. The pathological spate did not introduce any change into the peak region, and also did not alter the halfwidth of the zone, but significant changes in the luminescence intensity occurred: it increase in 1.5-2 times at the tuberculosis, and decrease in 1.4-1.8 times at the cancer. At this lengthy waves exactly the luminescence of ketonic compounds and in our system take place quadratic break chain [4].

The typical spectrum is presented on the figure 2.

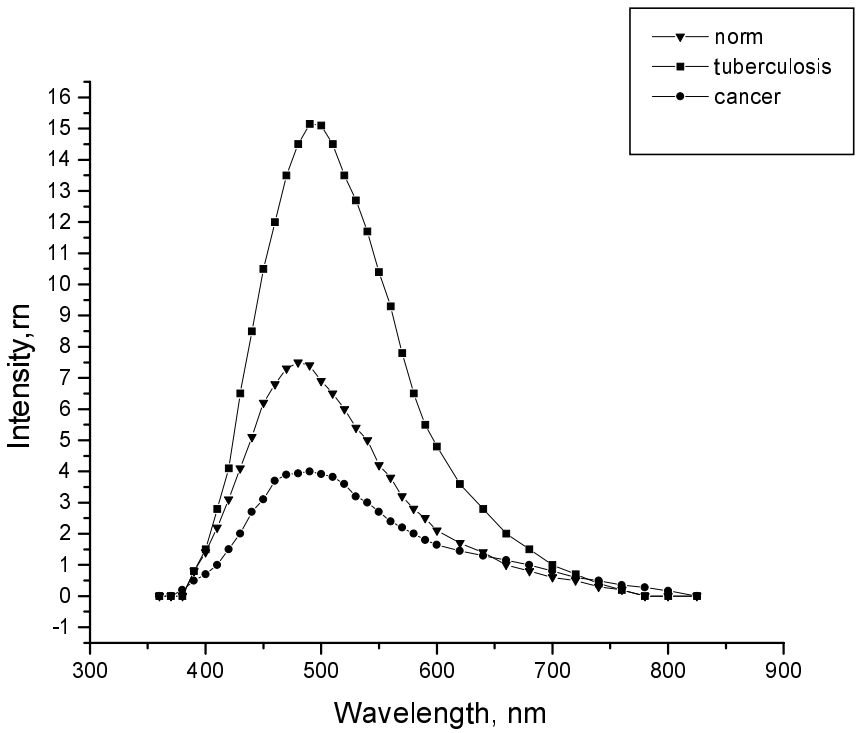

Fig. 2. Dependence of total chemilum inescence on the wavelength

In the next stage we investigated the lifetime of the excited state; it determines from whence takes place the quantum transitions takes place.

The basis of experiments was the change concentration $[H_2O_2]$ in the system [6], which has the influence on lighting effects. In case of addition reaction 0.1 ml blood serum and 0.1 ml hydrogen peroxide of different concentration was found out that: in the low-concentration range, namely 6 % H_2O_2, the CL is increasing. Under the condition of further increase of concentration, the process of initiation and appearing of free radicals began to compete with the process of their

disappearing or in other words H_2O_2 began to decrease CL. The reduction in chemiluminescence yield as a function of the probe concentration was described using a Stern-Volmer equation [5]

$$\frac{I_0}{I} = 1 + k\tau_0 C \tag{8}$$

where I_0 and I are the chemiluminescence in the absence and presence of H_2O_2, respectively, τ_0 is the unquenched lifetime, the k represent the rate constant, C is the bulk concentration of the H_2O_2 in the system (more 6 %).

The greater is the lifetime τ_0 of excited states, the greater is the probability of encounter with deactivating molecule.

On the base of angle definitely it was found out, that $k\tau_0$ lies in the range from $0.7 \cdot 10^3$ l/g-m to $1.4 \cdot 10^3$ l/g-m, where k - is the constant of interaction between H_2O_2 and activated molecule. In our case $k \sim 10^9$ l/g-m·s^{-1}

So $\tau_0 \sim 10^{-6}$s and it means that ketones are in triplet states. The main contribution in the process of CL has reaction (6). In blood serum is under the process of the quadratic break chain. The process of chemical reaction depends of transition state.

The difference in the energy of initial and transition states is equal to the energy of activation of this reaction. Accordingly CL also has to depend from the energy of activation.

For the quadratic break chain

$$I = \eta_{CL} k_6 [RO_2]^2 = \eta_{CL} w_i \sim \eta_{CL} k[BS] \tag{9}$$

where

η_{CL} – quantum yield CL,

w_i – rate of initiation chemical reaction,

k – constant of dissociation BS (the rate constant of the chemical reaction) .

Previously it was shown that rate constant chemical reaction satisfy an empirical equation with usually enter in the forms Arrhenius equation [3]

$$k = k_0 \exp(-E_a / RT) \tag{10}$$

Mentioned functional dependence of the rate constant on the temperature was characteristic feature for the majority chemical reaction (k_0—under the exponential, E_a has dimensions of the energy and was named the energy of activation. With increasing the temperature exponent $(-E_a/RT)$ decrease and value $\exp(-E_a/RT)$ snowball it being all the rather depressed larger the energy of activation

We can write the following equation:

$$\ln I = const - \frac{E}{R}\frac{1}{T} \tag{11}$$

So the angle of line, which describes the dependence of logarithm of intensity CL from reverse temperature, determines the energy of activation.

The experiment shows that I really is changing depending on the temperature change according to the exponential law. The results are shown on the plot.

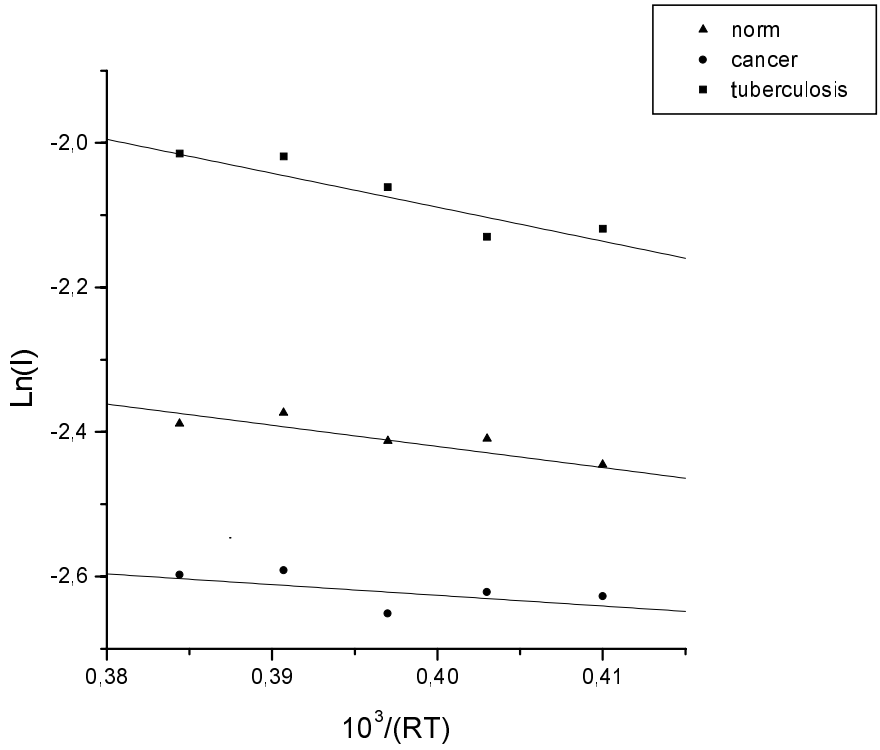

Fig. 2 Dependence logarithm of chemiluminescence on the reverse temperature

It was found out, that the energy of activation $E = 2.453 J/g - m$ in BS of healthy people and in case of oncological pathology, the energy of activation increases to $E = 3.086 J/g - m$, and in case of tuberculosis it decrease to $E = 1.874 J/g - m$. Increasing the energy of activation cancerous patient in comparing from norm maybe explained at the expense of increasing contents tocopherol in the BS. Formation lipids peroxides over a tocopherol may be entirely block[4]. In case of tuberculosis in BS leaving membranes bacterium of tuberculosis exist. This leads to increasing CL.

CONCLUSIONS

The main contribution into CL makes (6) reaction. The reaction products (6) can transform energy into the light more effectively compared to products (4) and (5) of reaction. When the concentration of H_2O_2 is increasing, the influence of (6) on the process of CL is increasing and lighting is also increasing. But in case of very high concentrations of H_2O_2 the lighting is competing with the processes of deactivation. It takes place under the condition of 7 (and more) % H_2O_2. It was found out that emitters for all pathologies are ketones in triplet states with survival time τ_p of excited states of around 10^{-6}s.

Currently there are not any theories about the distribution of complexes molecules, exothermal reactions products on different energy states. It is not known also what is the difference of chemical reaction energy transfer to one or another form of product energy (electronic, oscillating, rotational, translation)

The energy of activation of excitation triplet states is $E = 3.732\ J/g - m$ according to the norm. Depending of kind of decease it can change: in case of oncological disease it increases and in case of tuberculosis – it decrease.

So the determination of energy of activation can be used as additional differential-diagnostic test in case of cancer and tuberculosis pathology.

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THE APPLYING OF THE HYPER-TEXT MEANS FOR THE ELECTRONIC TECHNICAL PRODUCTS DESIGNING DOCUMENTS PRE-WORKING

Keywords: hyper-text system,designing documents

The electronic products designing documents hyper-text system structure, building principles, software and informational supplying are considered. By the specification record activising the system allows to find and load the appropriative software for the visualising and editing the designing document or product included by the electronic device.

1. INTRODUCTION

In the process of designing and technological pre-working of the electronic components production is used a large number of text and graphical designing documents: specifications, component lists, functional and principle electric schemes, drawings, miscellaneous datasheets and so on. When the traditional "paper" designing methodology is used, the search of necessary documents becomes a pretty timesinking process. In the "paperless" informational technology to minimize the documents search time the hyper-text scheme is purposed.