The Kinetics of Exogenous Phosphors Delayed Fluorescence in Tissues

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Abstract: The kinetics of delayed fluorescence (DF) and phosphorescence (Ph) of xanthene dyes in tumourous and normal mammary tissues of the BYRB line mice was investigated. Spontaneous mammary cancer tumours are characteristic of the mice of this line with the possible exogenous MMTV- retrovirus, found in leukocytic fraction.

The kinetics of DF and Ph molecules of the dyes was measured by means of laser flash-photolysis. The molecules in basic $S_0 \rightarrow S_1$ absorption band were excited by the second harmonic of pulsed laser to YAG:Nd^{3+} with wave length 532 nm. At the expiration of the process $S_1 \rightarrow T_1$ intersystem crossing (IC) and the formation of triplet T_1 molecule state, the registration system of delayed luminescence started. The luminescence was registered by PMT and monochromator. The parameters of the exciting pulse: pulse duration 10 ns, density of energizing power not more than 5 MWt/cm^2. The DF was registered at the wave length of 560 nm, and Ph at the wave length of 680 nm. The DF of the examined molecules can occur both as the result of termoactivated reverse to $T_1 \rightarrow S_1$ IC and as the result of the two T_1 states annihilation with the sequent formation of S_1 states. Moreover under the certain conditions in the presence of oxygen the cross – annihilation of the dye and oxygen stimulated molecules can occur.

It was demonstrated that the most effective quencher of the triplet T_1 states of phosphors in the tissues is the molecular oxygen $^3\Sigma_g^-(O_2)$. In the result of the interaction of the dye T_1 molecules with the molecular oxygen singlet $^1\Delta_g$ (O_2) oxygen is formed: $T_1 + {}^3\Sigma_g^-(O_2) \longrightarrow S_0 + {}^1\Delta_g(O_2)$. Then as the result of singlet-triplet $T_1 \rightarrow {}^1\Delta_g(O_2)$ annihilation of the rest triplet T_1 states of the dyes with $^1\Delta_g(O_2)$: $T_1 + {}^1\Delta_g(O_2) \longrightarrow S_1 + {}^3\Sigma_g^-(O_2)$ the singlet S_1 states of dyes are formed, thus contributing to the DF. As a result the registered kinetics of the DF of the dyes is made up of the three signals of various nature: termoactivated DF; T_1-T_1 annihilated DF; and luminescence due to the singlet-triplet $T_1 \rightarrow {}^1\Delta_g(O_2)$ cross–annihilation. The kinetic curve transforms and becomes no monotonously dependent on time.

It was specified that in the tissues during short periods of time the most significant contribution to the total signal was made by singlet-triplet $T_1 \rightarrow {}^1\Delta_g(O_2)$ annihilation. However, other things equal, the delayed luminescence kinetics in normal and pathogenous tissues differ. The contribution of the singlet-triplet annihilation to the total signal of the DF in the normal tissues is significantly less than in tumour, which indicates the less effectiveness of the triplet states of the dyed molecules interaction with the singlet oxygen.

Unlike DF the Ph intensity of dye molecules decreases monotonously with the period of time. Phosphorescence kinetics as well as DF differs within different tissues. The luminescence peculiarity depends on the phase of the tumour, the biotissue condition and other factors. However in all our experiments the common regularity is evident, namely the Ph lifetime in tumours is shorter than that in normal tissues.

Reliable registered differences in the dye tissue delayed luminescence kinetics can be used when developing an alternative method of optical diagnosis of biotissues. We assume that the method based on the measurement of lifetime of the delayed luminescence phosphor is fairly promising. Any combinations possessing delayed luminescence and meeting the requirements set to such specimen may serve as exogenous phosphor.

Keywords: Delay fluorescence, phosphorescence, optical diagnosis of tissues.

INTRODUCTION

It is possible to find out the pathology and get necessary information on it due to contact-free optical methods of bio-tissue diagnostics without doing any harm to the examined tissue. Laser fluorescent diagnostics (LFD) stands apart among the well-known optical diagnostic methods [1-2]. The LFD method is based on the comparative analysis of luminescent properties of endogenous or exogenous phosphors in the cells of normal and pathologic biological tissues.

The results of studying the peculiarities of delayed fluorescence (DF) and phosphorescence (Ph) kinetics of exogenous phosphors (organic dyes molecules) in bio-tissues are presented here. The possibility of application time emitting relaxation of phosphor excitation as the measurable parameter for bio-tissue diagnostics is being discussed [3-6].

RESEARCH METHODS AND SUBJECTS

The mammary tissues of both healthy and the BYRB line female mice with spontaneous tumors were under the examination [7]. The tissue pieces cut in the process of the operation were put into the aqueous solution of a dye with the initial concentration of 10^{-3} M

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and were kept there for 3-4 minutes. The dyed tissues were put into the hermetic thermostatic chamber. There were six different samples at a time. Therefore, the similar conditions for all the samples were created, thus providing the results to be valid. The gas content of atmosphere over the dyed tissues surface was changed by gaseous nitrogen venting of the chamber.

The tissues were dyed by erythrosine or eosin. The choice of dyes was determined by their spectral and luminescent properties: high quantum yield into triplet state, intense DF and Ph, good dissolution in water and the ability to penetrate into the cells [8-9].

The kinetics of DF (λ_{max}=570 nm) and Ph (λ_{max}=680 nm) of dye molecules after bio-tissue pulse photo-excitation were measured in the experiment. To population S_1 states of molecules the second harmonic emitting of YAG:Nd^{3+} laser with the wave length λ = 532 nm was used. The parameters of excitation pulse were: pulse duration 10 ns, power up to 20 mJ. The delay luminescence kinetics was registered by monochromator MDR–41 and PMT-84 with the managed electrode. At the moment of sample excitation there was "locking" of PMT by supplying negative impulse to the managed electrode from impulse generator G5-54. Locking impulse was equal to -100 V. Laser triggering, registering system and PMT locking synchronization, as well as collection, accumulation and the initial processing of the experimental data were performed on the automated system.

THE RESULTS AND THE DISCUSSION

As a rule at the normal air (atmospheric) pressure it is hard to find DF or Ph of molecules in the liquid solutions because phosphor triplet states are quenched effectively by molecular oxygen. In the tissues there are other triplet state quenchers except oxygen that interact with dye molecules thus additionally reducing the probability of their delay luminescence. In spite of this fact we were able to register xanthene dyes DF and Ph in the mice mammary and muscular tissues and examine their peculiarities.

The power difference between singlet S_1 and triplet T_1 states in xanthene dyes is about 2800 cm^{-1} [10] and at room temperature effective reverse intersystem crossing $T_1 \rightarrow S_1$ takes place resulting in termostimulated delay fluorescence (TDF) $S_1 \rightarrow S_0 + h\nu$. This monomolecular process and registered kinetic curve make up an exponent.

If molecules can move in the medium, then annihilation of two triplet T_1 excitations is possible. In case of creation of triplet-triplet pair there is a possibility of its break-up with the formation of S_1 state and consequent DF $S_1 \rightarrow S_0 + h\nu$ of annihilation type. The lifetime of such a luminescence is two times shorter than TDF.

Triplet phosphor quenching by molecular oxygen $^3\Sigma_g^-(O_2)$ is accompanied by photosensibilized generation of singlet $^1\Delta_g(O_2)$ oxygen $T_1 + {}^3\Sigma_g^-(O_2) \longrightarrow S_0 + {}^1\Delta_g(O_2)$. It should be noted that the concentration of T_1- excitation decreases and the reduction of luminescence intensity is to be expected. However, as it was shown in [11-13] during annihilation of migrating singlet oxygen with dye molecules that stayed unquenched, singlet S_1 phosphor state $T_1 + {}^1\Delta_g(O_2) \longrightarrow S_1 + {}^3\Sigma_g^-(O_2)$ additionally contributing to DF can form. Thus in this case the registered kinetics is made up of TDF as well as luminescence determined by the singlet-triplet and triplet-triplet annihilation. As a result the kinetic curve becomes non-monotonically dependent on time. At short periods of time the singlet-triplet annihilation contributes the most into the signal and the curve has a segment of DF intense increase.

It is known [14] that in tissues xanthene dyes color acidophilic and oxyphilic structures – cell cytoplasma, collagenous, elastic fiber, intracellular and extracellular proteins, and can be found both in free condition and bound state. Thus all above mentioned processes take place in the tissues. To illustrate this, erythrosine DF kinetics in the mouse mammary tissue under various oxygen concentration in atmosphere is shown on Figure **1**.

The oxygen concentration was varied by dosed nitrogen venting of the chamber with samples. At the span of 0 to 10 μs DF kinetics is a superposition of three signals of various natures. The most intensive signal (curve 1) was registered at air pressure in 2 μs after PMT "unlocking". Intensity increase is determined by singlet-triplet annihilation contribution into the total signal. This statement is easily proved by the experiment. After nitrogen venting the chamber with the samples, the kinetic curve becomes monotonically decay (curve 4).

On assuming that oxygen molecules migration in the cells is effective, a formally kinetic approach can be used to describe experimentally registered curve in mathematic terms. The intensity of DF molecules is described by the formula

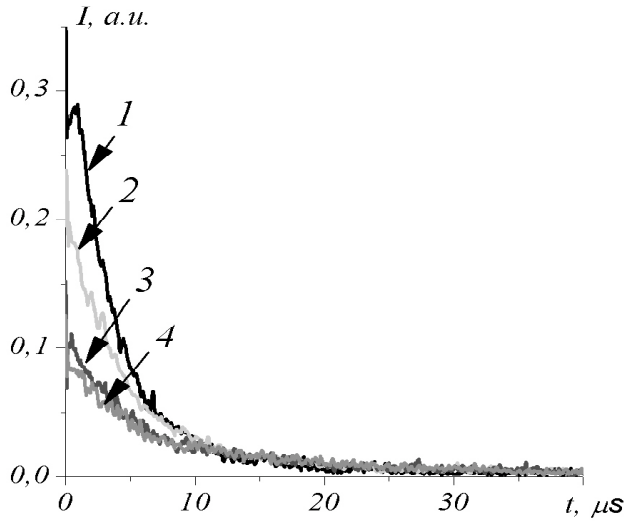

Figure 1: Erythrosine delayed fluorescence kinetics in mouse mammary tissue under various oxygen concentration in atmosphere:
1 – at atmospheric pressure; 2 – at nitrogen venting during 1 sec.; 3 - at nitrogen venting during 2 sec.; 4 - at nitrogen venting during 10 sec.

$$I_{DF}(t) = \varphi_{fl} p_S k_1 N_T(t) N_\Delta(t) + k_2 N_T^2(t) + \varphi_{fl} k_3 N_T(t) \quad (1)$$

The first summand in this equation determines luminescent component, conditioned by singlet-triplet annihilation, second – triplet-triplet annihilation, and third addend – thermostimulated delayed fluorescence.

In the equation (1) k_1 and k_2 – second-order rate constants determining singlet-triplet and triplet-triplet dye molecules annihilation respectively, k_3 – reverse $T_1 \rightarrow S_1$ intersystem crossing rate constant, φ_{fl} - fluorescence quantum yield, p_S - the possibility of S_1 molecules state formation in annihilation reaction $T - {}^1\Delta_g$. The kinetics $I_{DF}(t)$ at short periods of time is mainly determined by multiplier $N_\Delta(t)$ in annihilation component, because the contribution of the others addends at this segment is relatively small.

To find out $T_1 - T_1$ annihilation contribution to the registered signal, dye DF kinetics in tissues in nitrogen atmosphere was studied. It is worth noting that we couldn't receive absolutely coincident curves for various mice, but general regularity was established. In different samples the kinetic curve in semi-logarithmic coordinate is approximated by two lines. Thus on Figure **2** DF kinetics with fading constants $k_1 = 0{,}131 \cdot 10^6$ c^{-1} and $k_2 = 0{,}07 \cdot 10^6$ c^{-1} respectively are shown. In average, constant ratio k_1/k_2 is approximately 2 ÷ 2,5. This allows us to make an assumption that triplet-triplet annihilation process is developed in bio tissue cells. In liquid cell cytoplasm such process is quite possible. At time spans above 10 μs only TDF is registered as it can be seen on the Figure **2**.

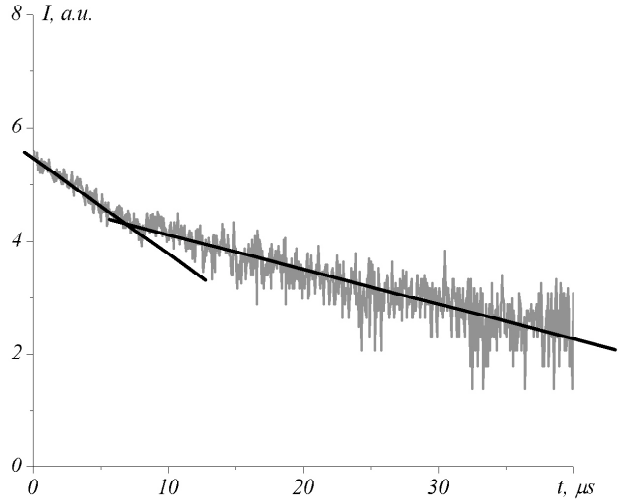

Figure 2: Eosin delayed fluorescence kinetics in mouse mammary tissue in nitrogen atmosphere.

It was found out that all other factors equal in normal and pathogenic tissues the luminescence kinetics is different. The contribution of singlet-triplet annihilation into the total DF signal in normal tissues is less than in tumourous ones thus making it evident that the effectiveness of dye molecules interaction with oxygen in them is less. Figure **3** shows the fragments of erythrosine DF kinetics in normal tissue and mouse mammary tissue tumor under normal atmospheric pressure. As a rule, luminescence intensity in pathogenic tissues is higher than in normal ones, and DF duration is on average 15-20 % less.

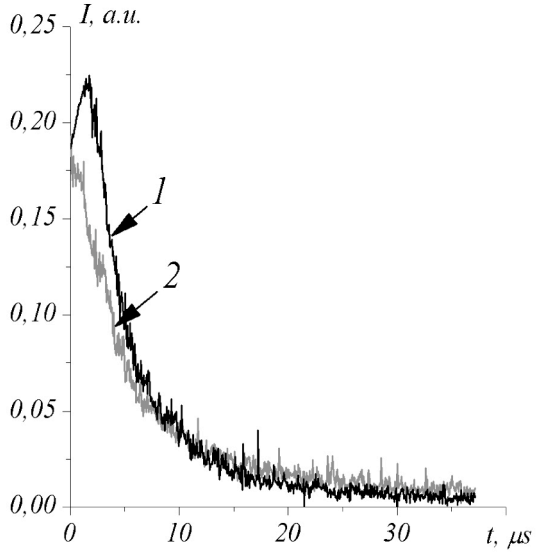

Figure 3: The fragments of erythrosine delayed fluorescence kinetics in tumor (1) and normal (2) tissue under normal atmospheric pressure.

In contrast to delayed fluorescence phosphorescence intensity of dye molecules monotonically changes at amplitude in the course of time. Figure **4** shows erythrosine delayed phosphorescence kinetics in normal and pathogenic tissues under normal atmospheric pressure. Kinetic curves are described by the following function:

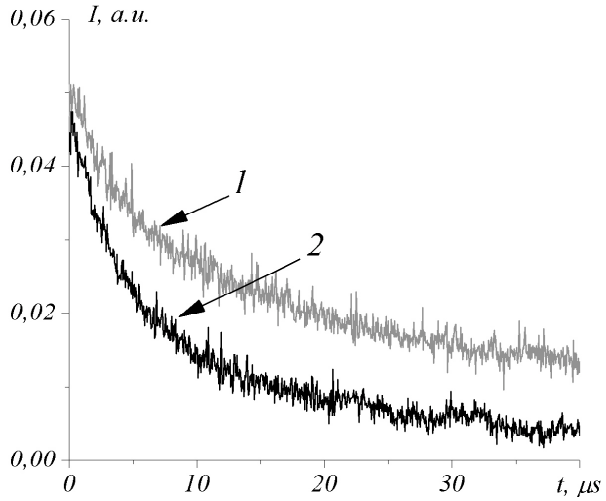

Figure 4: Erythrosine phosphorescence kinetics in timorous (1) and normal (2) tissues.

$$I_{Ph} = A_1 \exp(-t_1 / \tau_1) + A_2 \exp(-t_2 / \tau_2)$$

For the samples kinetics of which is shown on Figure **4**, the values of τ_1 and τ_2 in timorous tissue are equal to 6,94±0,39 μs and 47,23±3,93 μs, while in normal tissue 7,89±0,58 μs and 57,3±5,99 μs respectively.

Depending on tumor development stage, the ratio of lifetime in tissue, obtained from different mice varies. But in general the regularity is preserved – phosphorescence lifetime in tumor is always less than in normal tissue.

CONCLUSION

The established differences in delay phosphor luminescence kinetics in tissues can be applied for tissues diagnostics. Diagnostics method based on measuring delay phosphor luminescence lifetime in comparison with measuring fluorescence intensity or light dispersion is more advantageous. Thus, the information about absorption characteristics and light dispersion of the substances under research, as well as about fluorescent combination concentration in different tissues is not necessary for kinetic measurement as opposed to spectrophotometric measurement. Such information is difficult to be gained. High sensitivity of DF and Ph kinetics to environment changes will make possible to discover pathologic changes in cells at the very early stage. Any dyes possessing delayed luminescence and meeting the requirements set to such preparations can be used as exogenous phosphors.

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