PECULIARITIES OF THE DNA – STYRYLCYANINE DYE SYSTEM LUMINESCENCE

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Fluorescence and phosphorescence of styryl dye Dbos-30 in the free state and in the presence of deoxyribonucleic acid (DNA) in the temperature range of 77–293 K are investigated. At a temperature of 77 K, the fluorescence and phosphorescence of both single chromophores and dye aggregates non-fluorescent at room temperature are registered. It is shown that there are two centers of phosphorescence in the frozen solution of Dbos-30: single chromophores and molecular aggregates. In a mixed dye-DNA solution, the quenching of the DNA macromolecule phosphorescence is observed. Furthermore, the phosphorescence and delayed fluorescence of the dye upon the excitation in the absorption band of DNA are registered. This is the evidence of the triplet electronic excitation (TEE) energy transfer from DNA to the probes bound to this macromolecule and the annihilation of such mobile T-excitations on these dyes. It is ascertained that the average mobile TEE displacement along a DNA macromolecule l_T amounts to about twenty base pairs, which confirms the estimations made using another methods [1, 2].

1. Introduction

Fluorescent probes are widely applied in the detection and the imaging of the localization places of nucleic acids (NA) in living cells. Such methods are based on the changes of the spectral properties of probes while they bind with DNA (the fluorescence intensity of dyes increases up to 1000 times). For the successful usage of the novel probes, the detailed study of their peculiarities is necessary: positions of the energy levels, the variants of the electronic excitation deactivation, spectral manifestations of the interaction with NA, etc. One of the tasks posed in this work is the investigation of the peculiarities of the luminescence of dyes in the free state and in the presence of DNA at room temperature and at 77 K.

Small molecules which bind with biomacromolecules can be also used for studying the electronic processes which take place in biopolymers. One of such processes is the electronic excitation (EE) energy transfer.

It is known that DNA nucleotide bases absorb ultraviolet radiation as separated centers. Hence, electronic excitations are localized in this molecule [3]. Such excitations can lead to a damage of DNA: for instance, TEEs in the DNA are responsible for the initiation of photochemical reactions [4]. Therefore, the study of the behavior of these EEs and their characteristics is of extreme importance.

There is the field of investigations, in which the existence of the intramacromolecular migration of TEEs is demonstrated directly or indirectly [1–8]. To the estimation of such a parameter as the average mobile TEE displacement l_T, one can apply different methods which are joined by the idea to use traps for migrating TEEs. In [8], the extrinsic nitrated units were used as traps in a synthetic macromolecule of polyvinylcarbazole. Estimations of thel_T for the synthetic polymers in the temperature range of 4.2–300 K were made, by introducing the additional modified units into macromolecules and analyzing the interception of TEEs by molecular oxygen [7]. In [1], the DNA phosphorescence quenching by the paramagnetic ions bound to this macromolecule was investigated. The authors of work [2] proved that, in a DNA macromolecule, AT sequences (probably exciplexes) constituted from the neighbor A (adenine) and T (thymine) bases in the DNAs' line are the traps for mobile TEEs. With the known average rate of appearance of such AT sequences, the value of l_T was estimated.

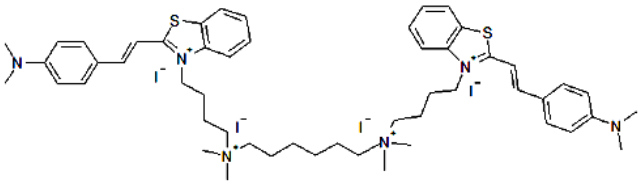


Fig. 1. Chemical structure of benzothiazole styryl Dbos-30

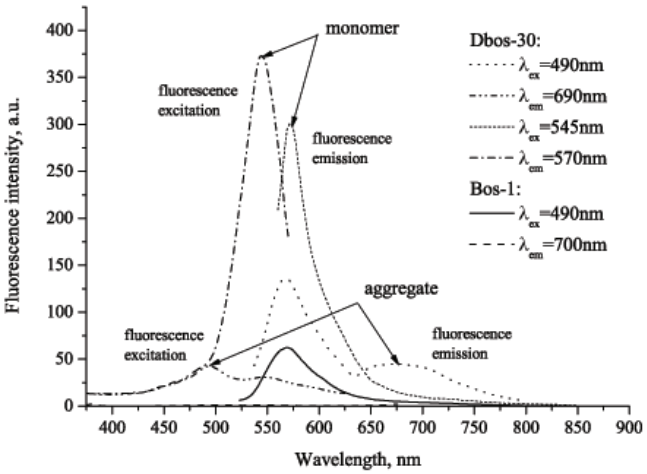


Fig. 2. Fluorescence emission and excitation spectra of a Dbos-30 dye solution in distilled water at $T = 77$ K; $c = 10^{-4}M$; $\lambda_{\rm em}$, $\lambda_{\rm ex}$ – wavelengths of fluorescence emission and excitation maxima, nm

In the current research, styrylcyanine dye Dbos-30 is used as a trap for mobile T-excitations for the first time (earlier, cyanine dye was used for the investigation of the migration of S-excitations [3]). We registered the manifestations of the existence of the nonradiative triplet electronic excitation energy transfer from DNA to the fluorescent probes bound to this macromolecule: 1) the quenching of the DNAs' phosphorescence on the addition of Dbos-30 dye; 2) phosphorescence and delayed fluorescence of probes upon the excitation at a wavelength of 260 nm (DNAs' absorption maximum), where the contribution of the dye to the total absorbance is negligible. The delayed fluorescence intensity increases with the exciting light intensity L in the next way: at low values of L, the dependence is linear, and it becomes close to the second-order one beginning from the certain value of L. This is one more evidence of the triplet electronic excitation energy transfer along a DNA macromolecule and its annihilation on the dye-traps bound with it.

2. Experimental

Benzothiazole homodimer dye Dbos-30 used as a trap for the above-mentioned mobile TEEs was designed and synthesized at the laboratory of the Combinatorial Chemistry department of the Institute of Molecular Biology and Genetics of the National Academy of Science of Ukraine. The chemical structure of the probe is given in Fig. 1.

DNA from chicken erythrocytes was purchased from Sigma. The concentrations of the substances in solutions were: 10^{-4} M of the dye and from 10^{-3} M of DNA base pairs (it corresponds to the ratio of one dye chromophore per ten base pairs of DNA) to 3×10^{-3} M of DNA base pairs (it corresponds to the ratio of one dye chromophore per thirty base pairs of DNA). In the preparation of solutions, distilled water or TRIS-HCl water buffer was necessarily used as a solvent.

The luminescence measurements were performed using a spectrofluorimeter Cary Eclipse (Australia) with a cryostat. The measurements of the absorption were made using a spectrophotometer Specord M40.

3. Results and Discussion

General spectral properties of dye DBos-30 at room temperature in the free state and in the presence of DNA were reported in [9]. In water solutions, the given styryl composes molecular aggregates of the H-type which disintegrate when binding with DNA and don't manifest themselves in the fluorescence emission and excitation spectra. The situation changes when the samples are cooled down to a temperature of 77 K. In the fluorescence excitation spectrum, the band blue-shifted relatively to the band of a monomer is observed (maximum at 495 nm, Fig. 2). It corresponds accurately to the band of intramolecular H-aggregates which is observed in the absorption spectrum at room temperature (492 nm, Fig. 3). Upon the excitation at a wavelength of 490 nm, a red-shifted (relatively to the band of a monomer) band with the maximum at 675 nm appears in the fluorescence spectrum. It is interpreted as the fluorescence band of aggregated molecules.

There are also the manifestations of aggregates of small molecules in the phosphorescence emission and excitation spectra (Fig. 4). In addition to the band of a single chromophore (maxima at 567 nm in the phosphorescence excitation spectrum and at 715 nm in the phosphorescence emission spectrum, mark A in Fig. 4), the band of associated molecules is present (maxima at 494 nm and 743 nm, respectively, mark B in

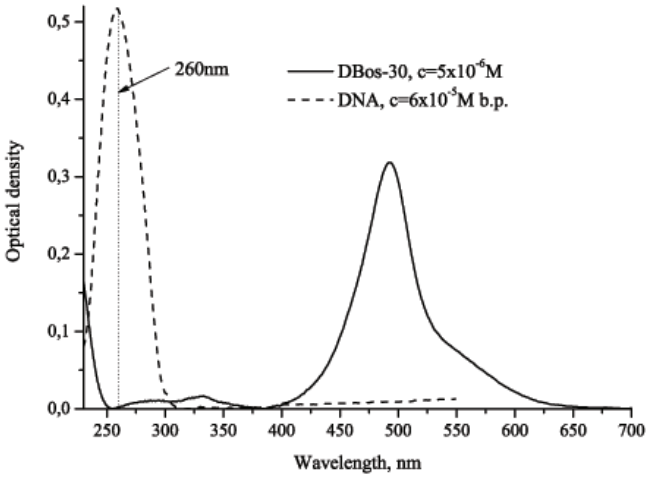


Fig. 3. Absorption spectra of dye Dbos-30 and DNA solutions in distilled water

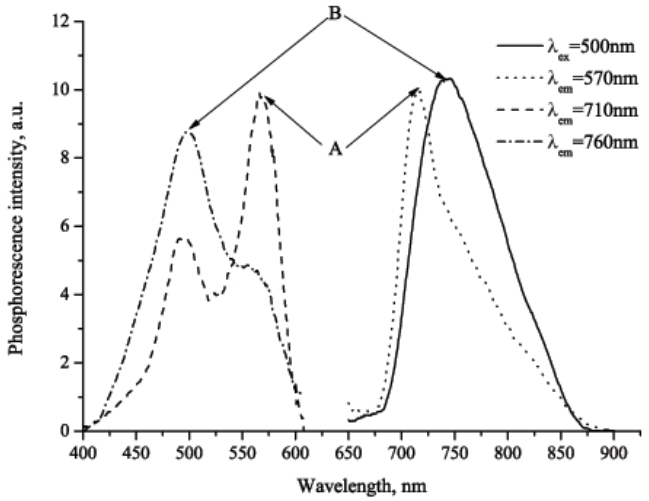


Fig. 4. Phosphorescence emission and excitation spectra of a dye Dbos-30 solution in distilled water (T = 77 K, $c = 10^{-4}$ M): A – band of a monomer, B – band of H-aggregates of dye molecules, λ_{em}, λ_{ex} – wavelengths of phosphorescence emission and excitation maxima, nm

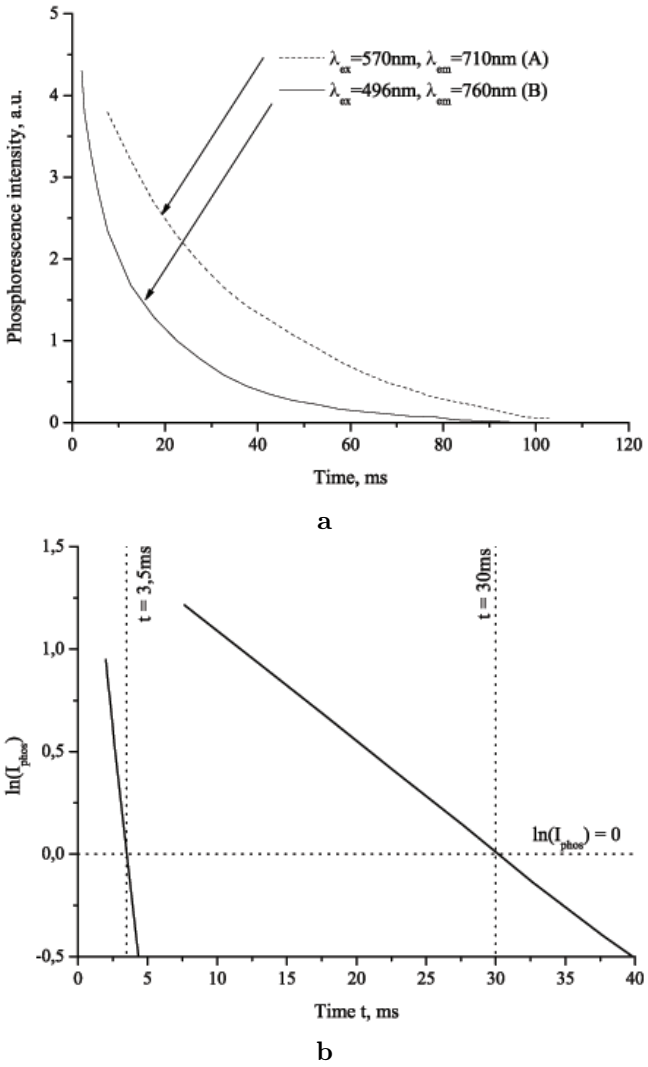


Fig. 5. Phosphorescence decay curves of homodimer Dbos-30: a – dependence of the phosphorescence intensity on the time, b – dependence of the logarithm of the phosphorescence intensity on the time. The wavelengths of phosphorescence emission (λ_{em}) and excitation (λ_{ex}) maxima correspond to: A – separated chromophores, B – intramolecular aggregates of the dye

Fig. 4). The phosphorescence decay curves which correspond to the emission of single chromophores (λ_{ex} = 570 nm, λ_{em} = 710 nm) and aggregates (λ_{ex} = 496 nm, λ_{em} = 760 nm) are given in Fig. 5,*a*. As it can be seen from Fig. 5,*b*, where the natural logarithm of the phosphorescence intensity versus the time is given, two centers of phosphorescence which are present in the solution demonstrate different lifetimes: the first corresponds to the phosphorescence of single chromophores (30 ms), the second – the phosphorescence of aggregates (3.5 ms). It is hardly possible to define the triplet level energy of a single chromophore T_1 and one of the intramolecular aggregates T_1' by defining the blue edges of phosphorescence bands, since the bands are overlapped and cannot be separated practically.

It was found out that, when adding dye Dbos-30 to a DNA solution, the quenching of the DNA phosphorescence occurs (band C in Fig. 6). DNA bases absorb as separated centers; thus, electronic excitations are localized. Singlet excitations as far as triplet ones can

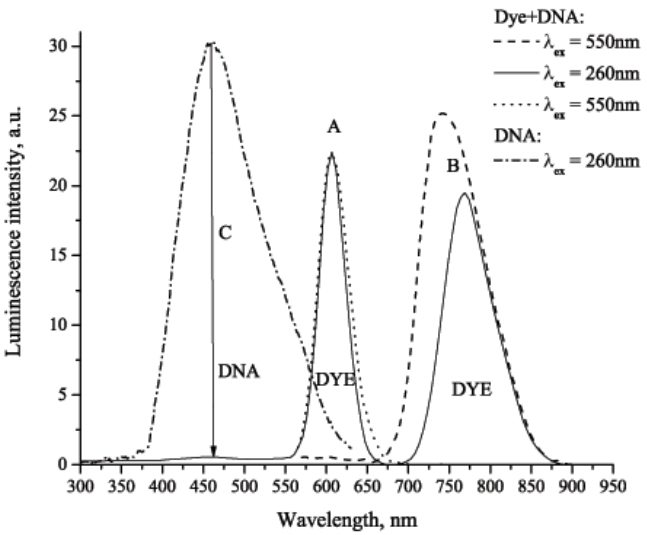


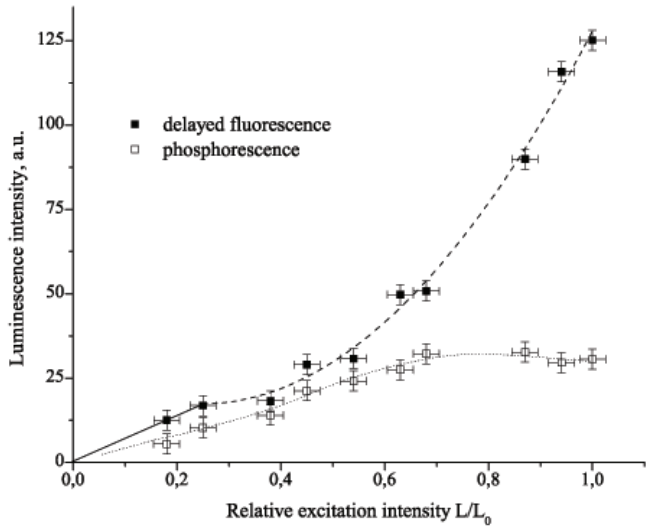
Fig. 6. Luminescence spectra of dye Dbos-30 in the presence of DNA at $T = 77$ K. Solid line: band A corresponds to the delayed fluorescence of the dye, band B – phosphorescence of the dye. Dotted line – normalized fluorescence spectrum of a dye-DNA solution excited at the own absorption maximum wavelength of the dye, dashed line – phosphorescence of the dye excited at the same wavelength, dash-dotted line – DNA phosphorescence without dye

migrate in a DNA macromolecule and can be captured by traps. Then the phosphorescence intensity of absorbing centers decreases, and the phosphorescence of traps occurs. The traps can be formed from the structural units of a DNA macromolecule (for example, AT sequences) [2] or can be foreign (in this very case – dyes bound to DNA). The quenching of the DNA phosphorescence is the proof of the existence of the transfer of T-excitations inside a DNA macromolecule, as well as from the DNA to dyes bound with it.

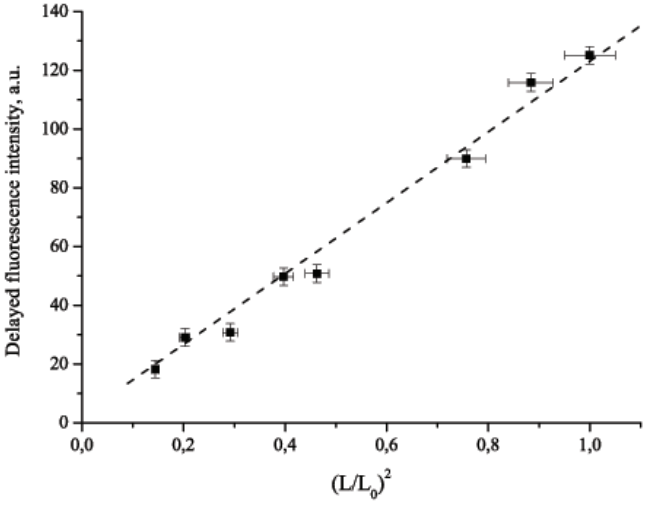
In Fig. 3, the absorption spectra of a Dbos-30 solution and a DNA solution are given. The concentrations were chosen in such a way that the case corresponded to the absorption of a mixed solution with the ratio between the number of dye chromophores and the number of DNA base pairs n equal to 1:10 (the highest value of n used in the investigations of the TEE energy transfer from DNA to the dye). As it can be seen from the spectrum given in Fig. 3 at a wavelength of 260 nm, the own absorption of the dye is negligible. The luminescence of the dye upon the excitation at the wavelength of the DNA absorption maximum is the one more evidence of the existence of the transfer of T-excitations from the biopolymer to a probe. The respective spectra are given in Fig. 6. We note that, by its shape and position, band A which was gained while recording the phosphorescence of the dye (the excitation wavelength is equal to 260 nm) coincides with the fluorescence spectrum of the dye gained upon the excitation at the wavelength of its own absorption maximum, but the lifetime of A amounts to 12 ms. Hence, band A is the delayed fluorescence of Dbos-30. Its intensity increase with the exciting light intensity L in the next way: at low values of L, the dependence is linear and, beginning from the certain value, becomes close to L^2 (Fig. 7,*a, b*). This is in agreement with the model of the annihilation of mobile triplet excitations on a trap proposed in [6–8] for the synthetic macromolecules. At the same time, the phosphorescence of the probes bound to DNA increases sublinearly (Fig. 7,*a*). The probable explanation of this fact is the domination of the annihilation of two mobile triplet excitations (and, as a result, the radiative transition $S_1 \to S_0$) upon the deactivation of the excitation through the emission from T_1. On the excitation at the own absorption maximum of the dye, no delayed fluorescence is observed (see the dashed curve in Fig. 6).

In the previous work [9], it was found that the number of aggregated chromophores decreases when binding with DNA. At the ratio between the number of dye chromophores and the number of DNA base pairs n equal to 1:10, only one phosphorescence center which corresponds to nonaggregated molecules is observed. So, by determining the blue edge of the phosphorescence band, it was evaluated that the triplet energy level T_1 of the dye bound with DNA amounts to 14840 cm^{-1}. This is essentially less than the energy of the lowest triplet level among DNA bases which is equal to 25950 cm^{-1}[2]. Probably, the act of the TEE energy transfer happens by involving the higher electronic triplet level of the dye T_2.

As was noticed above, dye Dbos-30 bound with DNA is a trap for the mobile triplet excitation which migrates along the DNA macromolecule. Let the distance between neighbor bound dyes, which is determined by the number N of DNA base pairs between them, become comparable with the average mobile TEE displacement in a DNA macromolecule, l_T. This situation corresponds to the saturation of the relative phosphorescence intensity of the bound dye upon the excitation at the DNA absorption maximum wavelength. In our studies, we used the samples with different ratios n [namely, 1:30, 1:22, 1:20, 1:18, 1:16, and 1:10 (respective values of N are 30, 22, 20, 18, 16, and 10 DNA base pairs)]. Analyzing the shape of the dependence given in Fig. 8, one can see that the sharp rise of the relative phosphorescence intensity (the excitation at a wavelength of 260 nm) stops near the value of n equal to 1/20 (N=20 base



a



b

Fig. 7. Dependence of the luminescence intensity of the dye bound to DNA on the relative excitation intensity L/L_0 (a) and on the square of the relative excitation intensity $(L/L_0)^2$ (b). The excitation wavelength is 260 nm; the ratio between the number of dye chromophores and the number of DNA base pairs n is equal to 1:10

pairs). With the further growth of the relative concentration of the dye (a decrease of N), the saturation is gained. Hence, the average mobile T-excitation displacement in a DNA macromolecule amounts to about 20 base pairs. In the literature, the following estimations of the average mobile TEE displacement in a DNA macromolecule can be found: the authors of [2] obtained the value more than 16 base pairs, and Bersohn and Isenberg [1] – more than 20 base

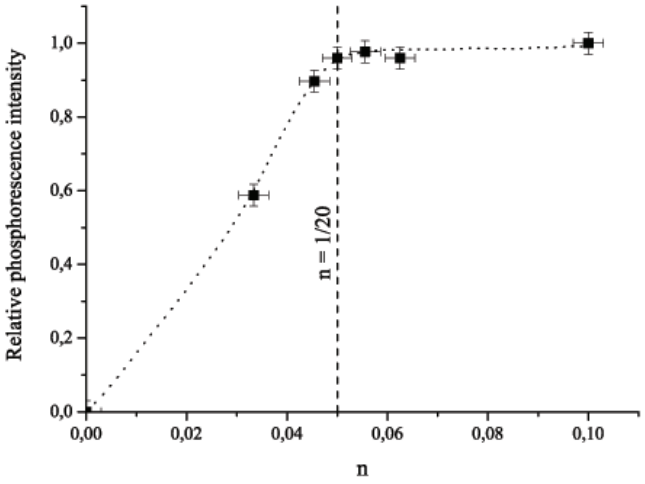


Fig. 8. Dependence of the relative phosphorescence intensity of dye Dbos-30 bound to DNA on the ratio n between the number of dyes chromophores and the number of DNA base pairs. The excitation wavelength is 260 nm, $T = 77$ K

pairs. The estimation of l_T made by us proves additionally the results of the researches mentioned above.

4. Conclusions

For the first time, we have registered the fluorescence of H-type aggregates of dye Dbos-30 molecules at $T = 77$ K which is red-shifted relatively to the fluorescence of a monomer, whereas no respective emission is observed at room temperature.

In a frozen solution of the investigated styryl dimer, two centers of phosphorescence with different lifetimes are present: separated chromophores (a lifetime of 30 ms) and molecular aggregates (red-shifted band, a lifetime of 3.5 ms).

The following manifestations of the existence of the nonradiative triplet electronic excitation energy transfer in a DNA macromolecule have been registered: a) the quenching of the DNA phosphorescence while adding dye Dbos-30; b) phosphorescence and delayed fluorescence of probes bound with DNA upon the excitation at the DNA absorption maximum; c) the delayed fluorescence intensity increases with the exciting light intensity L (firstly linearly and then, beginning from the certain value of L, as L^2, which evidences the annihilation of T-excitations on the dye-traps bound with DNA). At the same time, the phosphorescence of the probes bound to DNA increases sublinearly. The average mobile TEE displacement in a DNA macromolecule amounts to about twenty base pairs, which confirms

the estimations made with the use of the other methods [1, 2].

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ОСОБЛИВОСТІ ЛЮМІНЕСЦЕНЦІЇ СИСТЕМИ ДНК – СТИРИЛЦІАНІНОВИЙ БАРВНИК

В.П. Токар, М.Ю. Лосицький, В.М. Ящук

Р е з ю м е

Досліджено флюоресценцію та фосфоресценцію стирилового барвника Dbos-30 в діапазоні температур 77–293 K у вільному стані та у присутності дезоксирибонуклеїнової кислоти (ДНК). При температурі 77 K зафіксовано флюоресценцію та фосфоресценцію як окремих хромофорів, так і нелюмінесціюючих за кімнатної температури агрегатів барвників. Показано, що у замороженому розчині Dbos-30 присутні два центри фосфоресценції: окремі хромофори та молекулярні агрегати. У змішаному розчині барвник–ДНК спостерігалось гасіння фосфоресценції макромолекули ДНК. Крім того, під час збудження в області поглинання ДНК зареєстровано фосфоресценцію та затриману флюоресценцію барвника, що свідчить про перенесення енергії триплетного електронного збудження (ТЕЗ) від ДНК до зв'язаних з нею зондів та анігіляцію мобільних T-збуджень на цих барвниках. Встановлено, що середня довжина пробігу мобільного ТЕЗ в макромолекулі ДНК l_T становить близько двадцяти пар основ, що підтверджує оцінки, зроблені іншими методами в роботах [1, 2].