SPECTROSCOPIC STUDIES OF THE INTERACTION BETWEEN DNA AND FLUORENE DYES

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Making use of electron spectroscopy (absorption and fluorescence) and infrared vibrational spectroscopy methods, the interaction of the DNA taken from the normal and tumor (the yield strain of the Guerin carcinoma) tissues of rats of the Vistar line with two types of fluorene dyes, namely, (7-benzothiazol-2-yl-9,9-didecylfluoren-2-yl) diphenylamine (FD1) and {4-[2-(7-diphenylamino-9,9-diethylfluoren-2-yl)vinyl]phenyl} of the diethyl ester of phosphoric acid (FD2), has been investigated. The addition of the dyes to the tumor DNA in the *in vivo* experiment has been shown to disrupt the DNA secondary structure and to induce the transition into a partially unpaired form. In the IR experiment, different linkages of both dyes with the sugar-phosphate DNA frame have been detected, but it did not result in the features of the fluorescence spectra in the FD1 case. The fluorescence spectra of the FD2 interacting with the reference DNA show two bands, in contrast to what is measured at the interaction with the tumor DNA. The appearance of the short-wave band may be stipulated by an essential reconstruction in the part of the dye molecules due to their interaction with DNA ones. Experimental results evidence for that the dyes link more efficiently to the reference DNA than to the tumor one, and that the FD2 dye with a strong acceptor group reveals an enhanced activity during the interaction. The *in vivo* experiment shows that additional sites appear in the dyes for their linkage with DNA.

Introduction

The interaction between DNA and dyes and the study of their fluorescent properties are in the focus of investigators' attention since the sixties, when luminescence has been discovered for the first time in ethidium bromide at its intercalation into DNA [1]. Since then, it became a test reaction to reveal the double stranded DNA. Seeking for new effective probes to determine conformational states of biological molecules, in particular, DNA, and defects in them, is a very challenging problem both for fundamental science and from the aspects of their use in an early diagnostics of various diseases. Today, the probes are known on the basis of monomethine cyanines, in particular, thiazol orange (TO) [2] and oxazol yellow [3]. These are the so-called "flash probes" which are characterized by a substantial enhancement of the fluorescence quantum yield when bound to the DNA double helix. TO exhibits the specificity to the nucleotide sequence, namely, it links highly effectively to the double stranded DNA, interacts 5 to 100 times as less with the single helix of polypurines, and 10 to 1000 times as less with the single helix of polypyrimidines. Moreover, TO binds to the double stranded DNA and poly(dA) [it is a single stranded polynucleotide consisting of adenine and desoxyribose; similarly, poly(dG), poly(dC), and poly(dT) consist of guanine, cytosine, and thymine, respectively, and desoxyribose] as a monomer, to poly(dG) as a monomer and dimer, and to poly(dC) and poly(dT) mainly as a dimer. It is of interest that the TO solution, as well as the solution of ethidium bromide, has a very low luminescence quantum yield, but when bound to DNA, gives an intense luminescence. It occurs due to the relevant rotation restriction of aromatic rings, which closes the non-radiation channel of decay.

In the case of the DNA interaction with acrydine orange (AO) [4] and a number of other dyes, the luminescence wavelength depends on the DNA conformations, i.e. AO gives the green luminescence when interacting with the DNA double helix (in this case, AO interacts as a monomer with the DNA according to the intercalation type), and the red one when interacting with the single helix of RNA or DNA (here, AO, as a polymer, composes a complex with RNA or a single stranded DNA) [5].

The aim of this work is a complex investigation of the DNA interaction with fluorene dyes which can be proposed as a new class of fluorescent probes. This type of organic molecules is known to have a large cross-

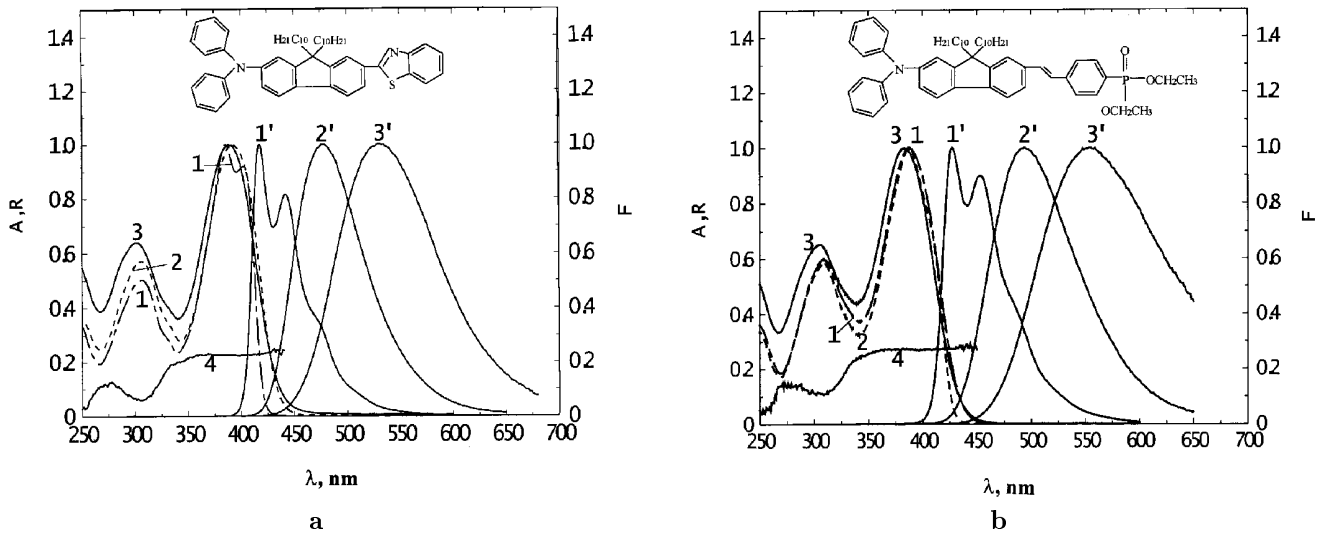

Fig. 1. Structural formula and the spectra of absorption (*1*, *2*, and *3*), fluorescence (*1′*, *2′*, and *3′*), and anisotropy (*4*) of FD1 (panel *a*) and FD2 (*b*) in hexane (*1* and *1′*), tetrahydrofurane (*2* and *2′*), and acetonitrile (*3* and *3′*). The anisotropy spectrum (*4*) was measured in silicon oil

section of two-photon absorption, which makes it possible to enhance the effectiveness of their practical implication for noninvasive fluorescent microscopy [6].

1. Materials and Methods

1.1. Dyes and their Spectral Properties

The nonsymmetric fluorene dyes with various electron-donor properties were used in experimental researches. Their molecular structure is outlined in Fig. 1. The synthesis of these compounds, namely, FD1 and FD2, was described in [6,7]. An element analysis gave the values greater than 99.6% for their purity degrees. As will be shown below, the existence of the strong acceptor group in FD2, the diethyl ester of phosphoric acid, leads to the enhanced interaction of the dye with DNA molecules.

The absorption spectra of all investigated specimens in UV- and visual-light ranges were obtained on a Cary-3 spectrophotometer. The spectra of stationary fluorescence, excitation, and excitation anisotropy were measured on a PTI Quantamaster spectrofluorimeter. The spectra of the excitation anisotropy for the dye solution were obtained making use of three polarizers in the T-format method [8]. Those measurements had great importance for the study of origins of the absorption spectra of FD1 and FD2.

The fluorescence lifetimes of the considered specimens were measured with a PTI Timemaster spectrofluorimeter at the nitrogen laser excitation (the wavelength $\lambda = 337$ nm) of the dye with $\lambda = 380$ nm. The software of the spectrofluorimeter allows one to obtain a time resolution of 100 ps. The spectral-luminescent properties of dyes FD1 and FD2 studied in this work, were partially described in our previous article [9]. In Fig. 1, the absorption, fluorescence, and excitation anisotropy spectra of those dyes are shown. The experimental data indicate that the maxima of the absorption bands and their halfwidths are determined mainly by the electron nature of end groups and are almost independent of the solvent polarity. The mole extinction coefficients of FD1 and FD2 in various solvents equal $(6 \div 8) \times 10^4$ $M^{-1}cm^{-1}$. The availability of the acceptor group of the diethyl ester of phosphoric acid in FD2 results in a shift of the absorption maximum by 12—17 nm towards the long-wave range. In contrast to the absorption spectra, the fluorescence ones manifest a strong dependence on the solvent polarity. This well-known solvation effect [8] is connected to the reorientation of the solvent shell around a dye molecule in the excited state. It is known [8] that the solvent polarity Δf can be expressed as

$$\Delta f = \frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}, \qquad (1)$$

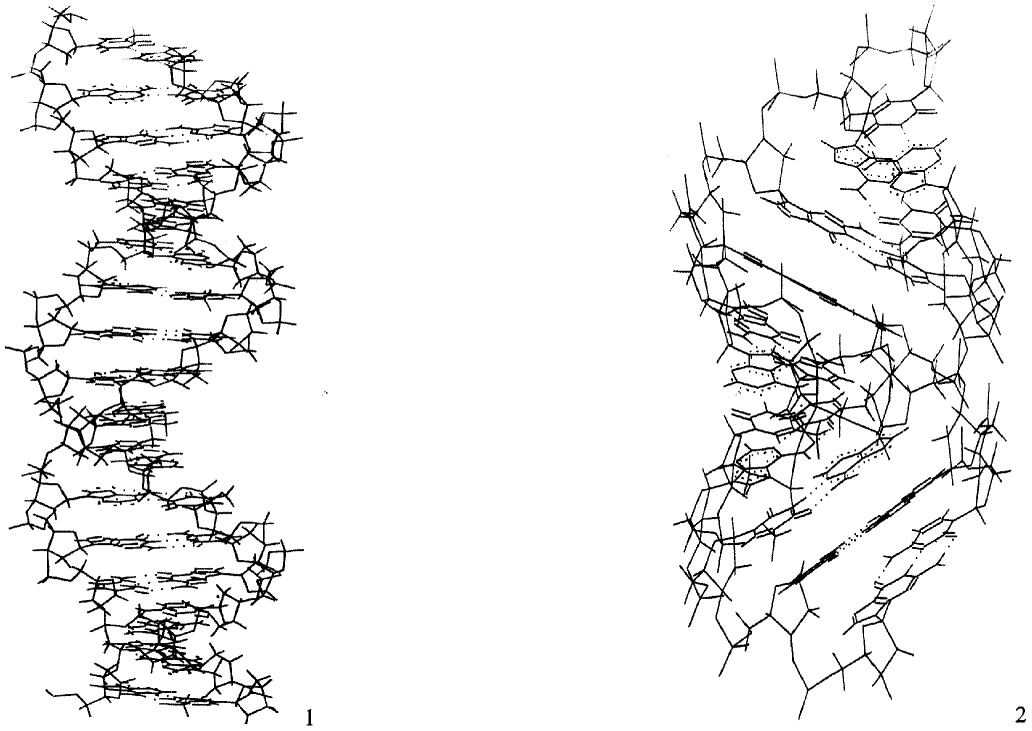

Fig. 2. Spatial structures of the DNA conformations: *1* — *B*-conformation, *2* — *A*-conformation

where ε and n are the dielectric constant and the refractive index of the solvent, respectively. The Δf-value affects the Stokes shift between the maxima of absorption and fluorescence contours. In a low-polar hexane ($\Delta f = 0.0012$), this shift is minimal (32–40 nm), and the fluorescent spectra reveal the vibronic structure. In more polar solvents, tetrahydrofurane ($\Delta f = 0.207$) and acetonitrile ($\Delta f = 0.257$), the fluorescence spectra have the form of broad non-structured bands with much higher Stokes shifts (up to 170 nm).

In Fig. 1, the excitation anisotropy spectra of the dyes in silicon oil are also presented. Everybody knows that measurements of anisotropy should be carried out in a solvent with a large viscosity to avoid the processes of deorientation of excited molecules. The spectral range with the wavelength $\lambda \geq 340$ nm corresponds to a constant anisotropy, which is connected to the existence of only one electron transition in this range. For $\lambda \leq 340$ nm, the decrease of the anisotropy values for both dyes is observed. The comparison of the spectral changes in the anisotropy and of the absorption spectra positions makes it possible to attribute the long-wave bands with $\lambda \geq 340$ nm to the first electron transition, and the short-wave bands with $\lambda \leq 340$ nm to the electron transition into the second excited state. Therefore, two bands in the absorption spectra correspond to two different electron transitions.

1.2. Spectral Properties of DNAs from Normal and Tumor Tissues

It is known that, spatially, DNA is a double helix possessing a flexible and variable structure shown in Fig. 2. DNA is characterized by a number of conformational states which are different according both to the helix type (the right-helix forms *A*, *B*, *C*, *D*, *E*, etc. and the left-helix one *Z*) and to the tilt angle of backbones relatively to the helix axis, as well as to the sugar conformation. In particular, a $C_{3'}$-*endo/anti* sugar conformation corresponds to the *A*-form of the DNA, and a $C_{2'}$-*endo/anti* one to the *B*-form [10]. "Anti" means such a base conformation relatively to a sugar where the base is directed out of the sugar, in contrast to the "syn"-conformation where the base is directed towards the sugar, hanging over it. The vibrational spectrum of nucleic acids is characterized by a high sensitivity to intra- and inter-molecular interactions, which allowed one, on the basis of the analysis of the normal vibrational modes in model compounds, to establish the correlation between the type of a helix

structure and the vibration frequencies of the relevant molecular groups (Fig. 3) [11].

The main markers of the DNA conformational state are the bands in the absorption ranges of backbones (1550÷1800 cm^{-1}), phosphates, i.e. the molecular groups PO_2^- (1000÷1350 cm^{-1}), and sugars (600÷1000 cm^{-1}). In particular, the band corresponding to antisymmetric vibrations of PO_2^- is situated at a frequency of 1220 cm^{-1} in the *B*-form and at 1240 cm^{-1} in the *A*-form. The symmetric vibration of the same molecular group together with $C_{5'}$—$O_{5'}$ valence vibrations are detected at 1085 cm^{-1} in the *B*- and at 1090 cm^{-1} in the *A*-form. The bands in the range 800—1000 cm^{-1} which are connected to the phosphodiester bond, together with the sugar and glycoside bond vibrations, are also sensitive to the conformational transitions in DNA. For example, the vibration at about 835 cm^{-1} is inherent in the *B*-geometry, while in the *A*-form there are two vibrations at about 805 and 860 cm^{-1}. Backbone vibrations manifest themselves in three different ranges: the in-plane stretching vibrations of the double links C=O and C=N between 1800 and 1550 cm^{-1}, the in-plane ring vibrations in the range of 1550–1100 cm^{-1}, and the non-in-plane ring vibrations at about 800 cm^{-1}. The band, which is a smoking gun of the DNA helix structure (it is absent in the denaturated DNA) and is connected to the valence vibrations of the backbone C=O molecular groups, is located at the frequency of 1715 cm^{-1} in the *B*-form and near 1705 cm^{-1} in the *A*-form [11—13].

In our previous works [14, 15], it was shown that the IR spectra of the DNA from tumor tissues are characterized by a number of peculiarities, namely,

(i) by a decrease, shift, and redistribution of the contributions of the integral intensities of components of the phosphate antisymmetric vibrations in the range of 1200—1300 cm^{-1};

(ii) by a separation as an isolated component of the C–O molecular group vibrations in the range of 1150 cm^{-1} and by a broadening of the PO_2^- symmetric-vibrations band;

(iii) by an availability of the marker absorption bands, which are attributed to various sugar conformations, and by a decrease of the sugar vibration intensity;

(iv) by the appearance of a great number of separated absorption bands in comparison with the reference specimen spectrum;

(v) by a redistribution of contributions between H-bonded OH and NH molecular groups and by an increasing of the integral intensity of valence vibrations of the CH groups;

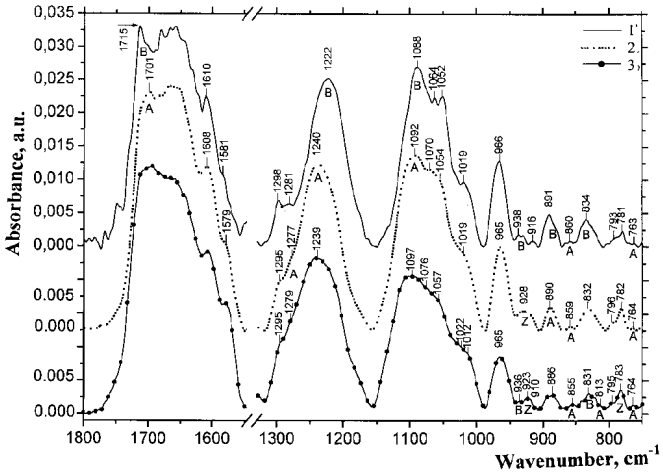

Fig. 3. SEIRA spectra of DNA: *1* — *B*-conformation, *2* — *A*-conformation, *3* — tumor DNA

(vi) by a 1.3—2-times decreasing of the integral intensity of the antisymmetric vibrations of the PO_2^- groups in comparison with the reference specimen;

(vii) by the appearance of the marker bands for $C_{3'}$-*endo* and $C_{2'}$-*endo* sugar conformations, which, together with the increase of absorption in the 1070 cm^{-1} range, evidences for the disorder enhancement in the tumor DNA structure.

1.3. Procedure of Preparing the DNA Specimens with Dyes in the in vitro and in vivo Experiments

Three animals (the rats of the genetically clear Wistar line), inoculated with the Carcinoma Guerin sensitive strain, were used in the experiment. Two animals were injected intra-abdominally by one of the dyes, FD1 or FD2, for the *in vivo* experiment. The amount of the injected preparation was 0.2 mg in 0.8 ml of CCl_4 on the basis of 5 mg per kg of the animal's mass (it was a single dose not resulting in the death of the animal during several hours). DNA specimens were isolated by the phenol method from frozen tumor tissues [16] in 1.5 hours after the dye injection, and from the lung tissue of the same animal for the reference. The third animal was not injected with dyes. DNAs, isolated from the tumor and lung tissues, were used in the *in vitro* experiment. After isolation, DNA was precipitated by the 70% alcohol solution and stored in a refrigerator. All steps of the procedures of the DNA isolation from tumor or normal tissues were identical, as well as the storage conditions. The specimen purity was checked

using the measurement of absorption in the range of 260—280 nm. The gel-electrophoretic studies did not reveal the presence of low-molecular fragments.

For carrying out the experiment *in vivo*, DNA specimens (of about 300 μg) were divided into two parts and dissolved in a small amount of water. To avoid denaturation, the aqueous DNA solution was prepared at the temperature 0 °C in an ice bath, with water being added in small portions: drop by drop, 20 μl each. Then, 40 μl of the dye FD2 alcohol solution or 80 μl of the dye FD1 alcohol solution (their concentrations were equal approximately to 10^{-5} M) were added to the gel-like DNA solutions of a concentration of about 1 mg/ml. Specimens prepared in such a manner were kept in a refrigerator during day and night. The following enumeration has been introduced for the specimens.

The experiment *in vitro*:

- specimen No. 1 — the reference DNA from lung tissue + FD1;
- specimen No. 2 — the reference DNA from lung tissue + FD2;
- specimen No. 3 — the DNA from carcinoma or tumor + FD1;
- specimen No. 4 — the DNA from carcinoma or tumor + FD2.

The experiment *in vivo*:

- specimen No. 5 — the reference DNA the lung tissue of an animal injected with FD1;
- specimen No. 6 — the DNA from carcinoma of an animal injected with FD2 or the DNA from the tumor after the FD2 action;
- specimen No. 7 — the DNA from carcinoma of an animal injected with FD1 or the DNA from the tumor after the FD1 action.

For the investigation of IR-absorption spectra, DNA in the aqueous solution was deposited on substrates and lyophilically dried. Glass plates, covered by a layer of sputtered gold 200—500 Å in thickness and with a surface roughness of about 50 Å , were used as substrates. IR-absorption spectra were measured by an IFS-48 Fourier spectrometer (Bruker, Germany) in the range of 380—5300 cm^{-1} in the reflectance mode. The wave number was measured with an accuracy of 0.01 cm^1, the resolution was 1 cm^{-1}, and the optical density was determined with an accuracy of 0.0005.

For the spectrum recording, a reflectance attachment was used with an incident angle of about 16.5°. Such a geometry of the experiment and the usage of rough metal substrates make it possible to detect the effect of the surface enhanced IR absorption (SEIRA) for nucleic acids [17]. Theoretical calculations of the enhancement factor for IR absorption give the value of 100; a 3 to 5-fold enhancement was detected in experiments for various vibrations [17]. The interpretation of the phenomenon is analogous to the effect of surface enhanced Raman scattering (SERS) which is known during last twenty years and can include two possible mechanisms: an enhancement of the external electromagnetic field in the vicinity of the rough metal surface due to the interaction with surface (local) plasmons, and a specific increasing of the dipole moments of molecule transitions at metal surface [18].

The evaluation of the spectra was made using an OPUS 2.2 computer program. Band positions were determined by the first-derivative method. For all spectra, a base-line correction was made, as well as a normalization according to the most intense band in the spectrum, i.e. the band of the stretching vibrations of OH or NH molecular groups in the range of 3300–3400 cm^{-1}.

2. Experimental Results and their Discussion

2.1. Vibrational Spectra

E x p e r i m e n t *i n v i t r o.* The analysis of IR-absorption spectra evidences for the interaction of dyes FD1 and FD2 with DNAs from both normal and tumor tissues which manifests itself in the absorption ranges of bases (1550—1800 cm^{-1}), phosphates (1000—1300 cm^{-1}), sugar moieties (600—1000 cm^{-1}), and H-bonds (2400—3800 cm^{-1}).

The addition of dyes FD1 and FD2 to specimens with the reference and tumor DNAs *in vitro* leads to essential changes in the structure of H-bonds, which is depicted in Figs. 4 and 5. It is evidenced for by a narrowing of the stretching vibration band of the H-bond OH and NH groups (by 150 cm^{-1} for the reference and by 300 cm^{-1} for the tumor) and by a decrease of the integral intensity of both the weak (3450—3800 cm^{-1}) and strong (3000—3400 cm^{-1}) H-bonds [19]. The dye influence on the tumor DNA results in a decrease by a factor of 1.6 of the halfwidth of the OH and NH bands in comparison with the tumor DNA without any dye, while for the reference DNA, the halfwidth of this band decreases by a factor of 1.1 after the interaction with

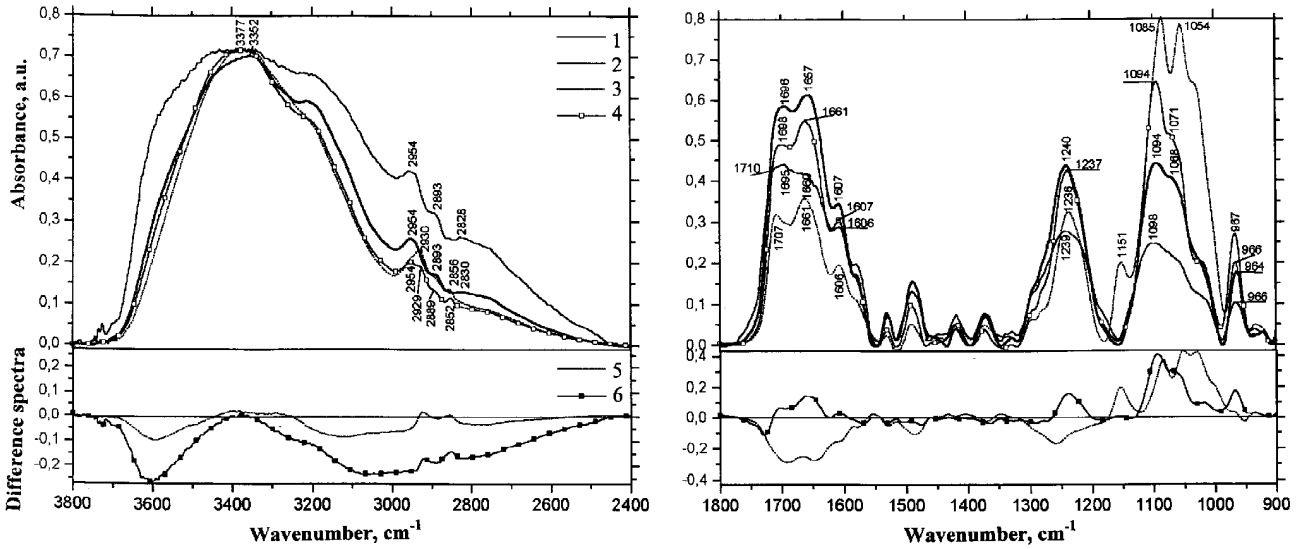

Fig. 4. Experiment *in vitro*. SEIRA spectra of DNA in the range 2400—3800 (panel *a*) and 900—1800 cm^{-1} (*b*): *1* — DNA from the animal lung injected by FD1 (specimen No. 5), *2* — tumor DNA without dye injection, *3* — reference DNA + FD1 (specimen No. 1), *4* — tumor DNA + FD1 (specimen No. 3). Differential spectra: *5* — reference DNA (specimen No. 1), *6* — tumor DNA (specimen No. 4)

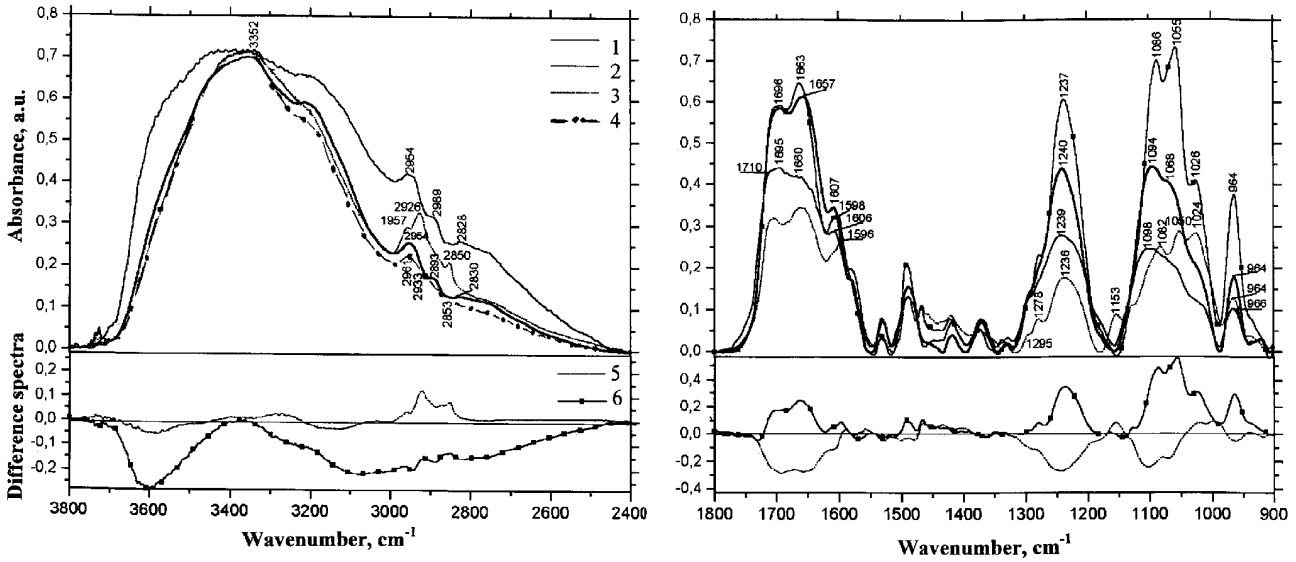

Fig. 5. Experiment *in vitro*. SEIRA spectra of DNA in the range 2400—3800 (panel *a*) and 900—1800 cm^{-1} (*b*): *1* – DNA from the lung of the animal injected by FD1 (specimen No. 5), *2* — tumor DNA without dye injection, *3* — reference DNA + FD2 (specimen No. 2), *4* — tumor DNA + FD2 (specimen No. 4). Differential spectra: *5* – reference DNA (specimen No. 2), *6* — tumor DNA (specimen No. 4)

dye FD1 and by a factor of 1.2 after dye FD2. Therefore, according to the spectroscopic data, the effect of the dye action is stronger for DNA from the tumor tissues, but it can be also a result of an essential difference between DNAs from the tumor and normal tissues from the viewpoint of their H-bonds (for example, the halfwidth of the OH stretching vibrations for the tumor DNA exceeds by a factor of 1.5 the relevant value for the reference DNA). An increasing of the intensity of CH stretching vibrations, in particular, of the CH_2 ones (2850 and

2920 cm^{-1}), in all DNA preparations with dyes may be due to an additive contribution of the absorption of the dye CH-group.

The suggestion about the rearrangement of H-bonding and hence the relevant changes in the DNA secondary structure finds its confirmation when analyzing the backbone absorption range, Figs. 4,*b* and 5,*b*. The intensity of the backbone band in the reference DNA after the dye action is almost half as large. The dye interaction with the tumor DNA led to an increase of the backbone intensity, which may indicate a destabilization of the DNA structure and its partial unpairing. With adding the dyes to the reference DNA specimens, a general tendency of the decrease of the halfwidth of the backbone absorption band (in the range of 1550–1800 cm^{-1}) was observed as well as the high-frequency shift of the strretching vibration band of the C=O backbone from 1656 cm^{-1} in the reference to 1662 cm^{-1}, i.e. by 7 cm^{-1}, which evidences for the rearrangement of the hydrogen pairing of backbones and the non-additive contribution of the dye absorption. (On the contrary, the simulation of a cumulative adding of the absorption intensities of the dye and the reference DNA gives rise to the increasing of the halfwidth of the backbone absorption band and to the low-frequency shift of the C=O band by 2 cm^{-1}.) The shift of the 1656 cm^{-1}-band may evidence for the increasing role of the position of the thymine $C=O_4$ group in the interaction of the reference DNA with the dyes. The dye interaction with the reference and tumor DNA specimens results in a decrease of the relative integral intensity of a shoulder in the range of 1715—1700 cm^{-1} (the stretching vibrations of the thymine C=O group, the band is a marker of the DNA conformational state [11, 12]), which indicates a break of the DNA helix structure.

An injection of the dyes to the reference DNA results in a drastic decreasing (almost by a factor of 2) of the integral intensity of the backbone absorption band in the range of 1550—1800 cm^{-1} (the 1.9 and 1.6 times decrease for FD1 and FD2, respectively) (see Figs. 4,*b* and 5,*b*). In the case of the tumor DNA, on the contrary, the increasing of the integral intensity of the backbone absorption band by a factor of 1.2 for both dyes is detected. Reviewing the results obtained, one may conclude that the addition of the dyes to the tumor DNA leads to a disruption of its condensed state and causes a DNA transition into a partially unpaired state. The FD2 dye stimulates this process more effectively, and deformation vibrations in the range 1300—1500 cm^{-1} verify it (see Fig. 5,*b*), while when FD1 is added, only initial changes are detected. The DNA of the normal tissue was initially in another structural form and partially unpaired, so its interaction with dyes runs more effectively. The appearance of covalent bonds between the dye and the reference DNA is probable, which leads to the decreasing of the backbone absorption band. The absorption in the range 1596 cm^{-1}, appearing after the addition of FD2, may be connected to the additive contribution of this dye absorption.

The most essential reconstruction of the spectrum takes place in the range of valence vibrations of the PO_2^- groups, 1000—1300 cm^{-1} (Figs. 4,*b* and 5,*b*). The increase of the integral intensity of the antisymmetric vibration band of the PO_2^- group for the tumor DNA is 1.1 times for FD1 and 1.6 times for FD2, and leads to the low-frequency shift by 4 cm^{-1}. For the reference DNA, the relevant values are 1.6, 2.8, and 5 cm^{-1}, respectively, which may be a result of the nonadditive contribution of the dye influence on the DNA structure (an additive summation of the DNA and dye spectra in this range gives a high-frequency shift of the main peaks and an increase of the vibration intensity).

In the sugar absorption range, FD2 induces a decrease of the main absorption bands both for the reference and tumor DNAs. On the other hand, on interacting with the reference DNA, the FD2 dye generates changes similar, according to their spectral manifestations, to the tumor DNA without dye, namely, the appearance of new conformational states for sugar and backbones. The FD1 dye induces minor spectral changes in this range, so one can suggest that it interacts weakly with sugars.

Thus, for the interaction of the DNA with dyes FD1 and FD2, a number of spectral changes are observed, namely, the decrease of the integral intensities of the backbone and phosphate bands, the decrease of their halfwidth, and the shifts towards the high-frequency range. The phenomena observed are often inverse to those which are produced by the additive summation of the DNA and dye spectra. The variations of band parameters under the influence of both dyes are approximately equal, but sometimes FD1 generates greater changes in comparison with FD2. In certain ranges, the inverse situation is observed. Nevertheless, the quality of the modifications in DNA shows that those dyes interact differently with DNA. We suppose that FD2, which stabilizes the DNA structure according to spectral data, inserts itself into the DNA structure, occupying the place in one of the grooves, or makes a covalent bond with various backbones or, alternatively, between backbones on the one side and sugars or

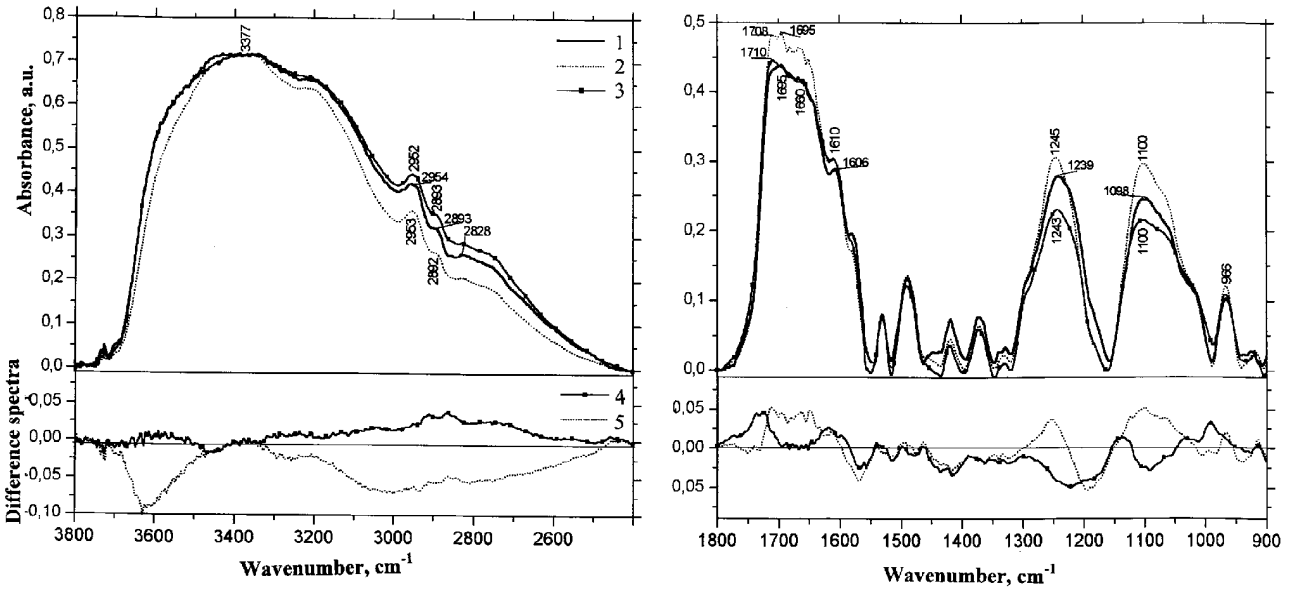

Fig. 6. Experiment *in vivo*. SEIRA spectra of DNA in the range 2400–3800 (panel *a*) and 900—1800 cm^{-1} (*b*): *1* — tumor DNA without dye injection, *2* — tumor DNA after FD2 injection (specimen No. 6), *3* — tumor DNA after FD1 injection (specimen No. 7). Differential spectra: *4* — tumor DNA (specimen No. 6), *6* — tumor DNA (specimen No. 7)

phosphates on the other side. As to FD1, it is bound, most probably, to phosphates or backbones without making up such bonds.

The IR spectroscopy data allow one to make a conclusion that dyes and antitumor preparations link better (their influence is stronger, or more linking bonds have the covalent character) to the normal tissue DNA than to the tumor one.

E x p e r i m e n t *i n v i v o*. The experiment carried out *in vivo* shows than DNA is also affected by the dyes. The IR spectra of DNA from tumor tissues after the intra-abdominal dye injection to the animals, have the typical view of the tumor DNA spectra, namely, the broadened range of the valence vibrations of the H-bond molecular groups OH—NH (the halfwidth is 600—700 cm^{-1}), the weakened phosphate and sugar bands, the peak value of the backbone absorption band is at 1708—1710 cm^{-1}, while, in the reference for DNA in the *A*-form (it is what is registered under our experimental conditions), it is at 1656 cm^{-1} (Fig. 6). Excess bands, connected to the explicit contribution to the absorption of the dyes themselves, were not detected. Spectral modifications consist in redistributing the absorption contributions of various molecular groups and in the variation of band intensities. No substantial frequency shifts have been found.

The FD1 dye does not induce essential spectral changes but the small (by 10 cm^{-1}) broadening of the valence vibrations band of the H-bond molecular groups OH—NH. On the contrary, FD2 narrows this band down to 591 cm^{-1} in comparison with the tumor case (708 cm^{-1}, see Fig. 6,*a*).

After the injection of both dyes, an insignificant growth of the integral intensity of the backbone absorption band and an increasing of the shoulder at 1700—1715 cm^{-1} are observed (Fig. 6,*b*). The shift towards high frequency is detected for the antisymmetric vibrations of phosphates from 1239 cm^{-1} in the tumor to 1243 cm^{-1} after the injection of FD1, and to 1245 cm^{-1} after the injection of FD2. No shift has been detected in the position of the PO_2^- symmetric vibration band. Concerning other parameters of the phosphate bands, the results are opposite for different dyes. While FD1 brings about to the decreasing of the integral intensity of the PO_2^- antisymmetric vibration band (from 22.8 in the tumor to 20.1 after the FD1 injection) and to the increasing of its halfwidth (from 86 cm^{-1} in the tumor to 91 cm^{-1} after the FD1 injection), FD2, on the contrary, stimulates the arising of its integral intensity and the diminishing of the halfwidth (23.6 and 77 cm^{-1}, respectively). Similarly, for the symmetric vibration, the integral intensity is 23.7 in the tumor DNA, 21.5 after the FD1 injection, and 29.2 after the FD2 injection. Moreover, the FD2 injection induces the redistribution of component contributions of this band in favor of the decreasing of the component at 1070 cm^{-1}. In the tumor

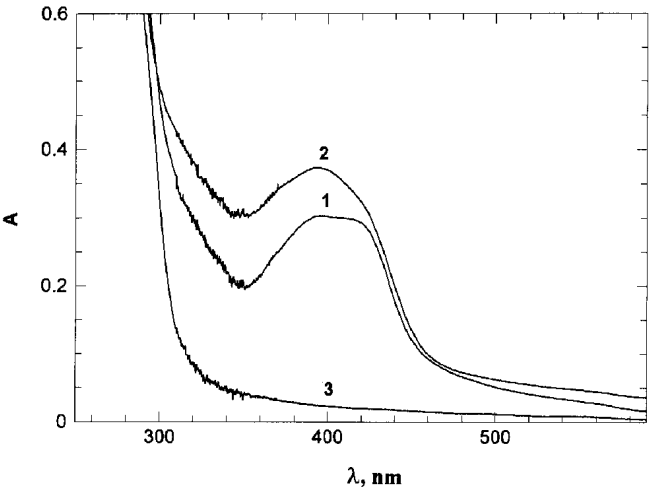

Fig. 7. Absorption spectra of the *in vitro* specimens for reference DNA + FD1 (specimen No. 1, curve *1*), reference DNA + FD2 (specimen No. 2, curve *2*), and of the *in vivo* specimen for tumor DNA + FD1 (specimen No. 7, curve *3*)

and in FD1, on the contrary, the relative intensity of this shoulder increases.

In the sugar range, vibrations are weakened. A substantial increasing of the integral intensity of the band at 860 cm^{-1} (the marker of the *A*-conformation of the DNA [12]) has been revealed. While the value of this parameter was 0.05 in the DNA from the tumor without dye injection, it equaled 0.1 after the FD1 injection, which is twice as large as the initial value. After the FD2 injection, it equaled 0.17, i.e. 3.4 times greater than the relevant value.

2.2. Absorption and Fluorescence Electron Spectra

In Fig. 7, the typical absorption spectra of the specimens *in vitro* No. 1 (the reference DNA + FD1), No. 2 (DNA + FD1), and the specimen *in vivo* No. 7 (the tumor DNA + FD1) are shown. An intense growth of the optical density in the range $\lambda < 300$ nm is connected to the DNA molecule absorption, which has the maximum at 260 nm and is characterized by a rather large molar extinction coefficient (about 5×10^4 $M^{-1}cm^{-1}$ [20]. For specimens Nos. 1 and 2, the absorption bands of dyes FD1 and FD2, respectively, are observed as well. The absorption by FD1 has not been revealed in the spectrum of specimen No. 7., which may be due to its much less concentration because of the insufficient accumulation in tumor tissues. The same is true for specimen Nos. 6 (the tumor DNA + FD2) and 5 (DNA of the lung tissue). The absorption spectra of FD1 and FD2 in DNA are deformed in comparison with those obtained in solutions. A negligible shift of the main absorption band towards the long-wave range and the flattening of its top are observed. The short-wave band of the dye absorption, corresponding to the second electron transition, overlaps with the own absorption of DNA molecules.

In Fig. 8,*a*, the fluorescence spectra of the reference and tumor DNAs *in vitro* (the specimens No. 1 and 3, respectively), activated by dye FD1 are depicted. The irradiation contours of both specimens are almost identical, with the maxima at 465 nm, and do not depend on the exciting wavelength. The spectral positions of the absorption and irradiation contours are most correspond to the FD1 spectra in tetrahydrofurane. That is, by analogy, one can evaluate the polarity of DNA as a solvation shell for FD1 in specimens Nos. 1 and 3. In accordance with our measurements, the DNA polarity is equal to that of tetrahydrofurane, which is characterized by the value $\Delta f \approx 0.2$.

The lifetime measurements of the FD1 excited state in specimens Nos. 1 and 3 revealed only a single component in the decay kinetics with a time constant $\tau = 0.5$ ns. Thus, one may conclude that, using FD1 as a probe of the DNA molecule state, the IR spectroscopy is a more sensitive method, which makes possible to detect more reliably the conformational changes and defects in DNA.

Let us consider the spectral-luminescence properties of the reference and tumor DNAs *in vitro*, injected by the FD2 dye (specimens Nos. 2 and 4, see Fig. 8,*b*). The fluorescence spectra of those specimens have great differences. If specimen No. 2 is excited by the waves with $\lambda = 290-330$ nm, the following bands are observed in its irradiation spectrum: a short-wave one with the maximum at about 350 nm and a band in the visible range with the maximum at 470 nm. The latter is an ordinary fluorescence of FD2 and its position is most corresponding to the FD2 spectrum in tetrahydrofurane. The origin of the short-wave band remains unclear. Most probable is the substantial variation of the dye structure due to its interaction with DNA. In so doing, a certain amount of FD2 might change its structure in such a way that the interaction intensity between the donor (diphenylamine) and acceptor (diethyl ester of phosphoric acid) parts of the FD2 molecule diminishes greatly, which results in a strong short-wave shift of the main absorption band and, respectively, of its fluorescence (for example, the long-wave absorption maximum of the non-substituted fluorene ≈ 300 nm [21]). The short-wave shift of the main absorption band

of the dye due to its interaction with DNA, may be also stipulated by the variation of the dye molecule geometry when the length of the π-electron conjugation reduces [22—24]. But, for fluorene dyes, the phenomena of this kind were not observed.

On the contrary, the fluorescence spectrum for specimen No. 4 includes only a single band in the visible range with the maximum shifted by 7—8 nm towards the long-wave direction in comparison with specimen No. 2. A certain difference between specimens Nos. 2 and 4 is also observed for the lifetime of the FD2 excited state, namely, 0.4 ns for specimen No. 2 and 0.3 ns for specimen No. 4. The availability of the single fluorescence band may indicate much less an interaction between FD2 and the DNA molecules in specimen No. 4.

The irradiation spectra of the *in vivo* specimens Nos. 5, 6, and 7 are more involved. First of all, it should be noted that the fluorescence of FD1 is observed even for the DNA taken from the lungs of the animals (specimen No. 5), despite that the solution of this dye was injected to the tumor localization site rather than to lung. The irradiation spectra of specimens Nos. 5 and 7 are shown in Fig. 8,*c*; they are similar and depend on the length of the exciting wave. In this case, the contour maxima are shifted from 350 to 420 nm.

As is seen, the spectra of the dye fluorescence in the *in vivo* specimens differ greatly from those of the *in vitro* ones. They are strongly shifted towards the long-wave direction and depend on the length of the exciting wave, which evidences for a nonuniform environment of dye molecules. The origin of those modifications can be connected to the enhanced interaction of the dyes with DNA molecules *in vivo*. For its investigation, additional experimental researches are needed. The lifetime measurements of the FD1 excited state in specimens Nos. 5 and 7 revealed the availability of a single component in the decay kinetics that possesses a lifetime $\tau = 1$ ns, twice as large as for the specimens *in vitro*.

An analogous structure of the fluorescence bands is observed for FD2 (specimen No. 6): the spectral maximum is shifted greatly towards the short-wave range and depends on the length of the exciting wave. The lifetime, in comparison with that in the specimens *in vitro*, also increases and attains the value $\tau = 1.4$ ns.

Thus, the interaction between DNA molecules and dyes for the *in vivo* specimens is essentially stronger. The increasing of τ may evidence for the partial inserting of dye molecules into the DNA structure.

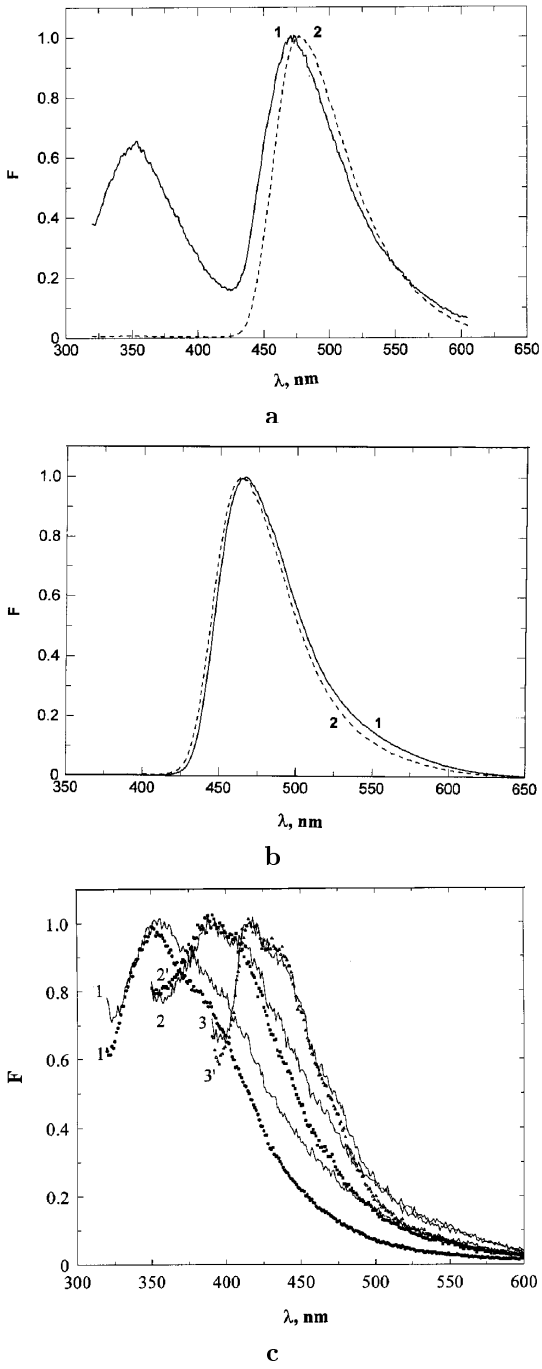

Fig. 8. *a* — fluorescence spectra of the specimens of the reference and tumor DNAs *in vitro* activated by FD2: *1* — specimen No. 2, *2* — specimen No. 3. *b* — the same as in panel *a* for specimens No. 1 (*1*) and 3 (*2*). *c* — fluorescence spectra of the specimens of the reference and tumor DNAs + FD1 *in vitro*: *1, 2, 3* — specimen No. 5, *1′, 2′, 3′* — specimen No. 7. The lengths of exciting waves: 310 nm (curves 1 and 1′), 330 nm (*2* and *2′*), and 370 nm (*3* and *3′*)

Conclusions

The process of interaction of DNAs from normal and tumor tissues with two types of the fluorene dye has been investigated. The luminescence evidences for that both dyes as early as in two hours after their injection into a tumor, appear in other organs.

The experimental data indicate that the dyes bind more effectively to the reference DNA than to the tumor one. In this case, the FD2 dye, which possesses the strong acceptor group of the diethyl ester of phosphoric acid, manifests a greater activity during the interaction.

The dye addition to the tumor DNA results in the disrupting of its secondary structure and induces the transition into a partially unpaired and disordered state.

The injection of the FD1 and FD2 dyes gives rise to a break of the helix structure both of the tumor and reference DNAs, as well as to their partial denaturation. In IR experiments, the linkage of both dyes with the sugar-phosphate backbone was detected, but it did not lead to the appearance of peculiarities in the fluorescence spectra for FD1.

In the FD2 fluorescence spectrum, providing the interaction with the reference DNA, two bands are observed contrary to the FD2 fluorescence spectrum when interacting with the tumor DNA. The short-wave fluorescence band may be connected to structure changes of the part of dye molecules due to the decreasing of the interaction between the donor and acceptor parts of an FD2 molecule. The ratio of the intensities of the short- and long-wave bands may indicate a deviation of the DNA molecule from the normal state. Therefore, we believe that further investigations can result in the developing of a new method of early diagnosis of the DNA structure modifications.

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СПЕКТРОСКОПІЧНІ ДОСЛІДЖЕННЯ ВЗАЄМОДІЇ ДНК З ФЛУОРЕНОВИМИ БАРВНИКАМИ

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Р е з ю м е

Методами електронної спектроскопії (поглинання, флуоресценції) та коливальної спектроскопії досліджено в експериментах *in vivo* та *in vitro* процес взаємодії ДНК з

нормальних та пухлинних тканин вихідного штаму карциноми Герена щурів лінії Вістар з двома типами флуоренових барвників: (7-бензотіазол-2-іл-9,9-дидецілфлуорен-2-іл)дифеніламіну, позначеного нами як ФБ1, та {4-[2-(7-дифеніламіно-9,9-діетілфлуорен-2-іл)вініл]феніл} діетілового ефіру фосфорної кислоти, позначеного як ФБ2. Показано, що в експерименті *in vitro* додавання барвників до пухлинної ДНК приводить до руйнування її вторинної структури і спричинює перехід у частково розплетений стан. В ІЧ-експерименті було зафіксовано неоднакове зв'язування обох барвників з цукрофосфатним остовом, але не було зафіксовано відповідних особливостей в спектрах флуоресценції ФБ1. Спектр флуоресценції барвника ФБ2 при взаємодії з контрольною ДНК показує дві смуги, на відміну від спектра, отриманого при взаємодії з пухлинною ДНК. Поява короткохвильової смуги може бути зумовлена суттєвою зміною структури частини молекул барвника внаслідок їхньої взаємодії з молекулами ДНК. Експериментальні результати свідчать про ефективніше зв'язування барвників з контрольною ДНК, ніж з пухлинною, причому барвник ФБ2 з сильною акцепторною групою виявляє більшу активність в процесі взаємодії. Експеримент *in vivo* свідчить про те, що з'являються додаткові місця зв'язування барвників з ДНК.