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Biophysics

γ-Irradiation Effect on DNA and Laser Induced FRET Method for Double Helix Quality Analysis

Vasil Bregadze[*], Zaza Melikishvili[**], Tamar Giorgadze[*], Shota Gogichaishvili[*], Mikheil Gogebashvili[*], Nazi Ivanishvili[*], Irine Khutsishvili[*]

[*] *Andronikashvili Institute of Physics, Ivane Javakhishvili Tbilisi State University, Tbilisi, Georgia*
[**] *Chavchanidze Institute of Cybernetics, Georgian Technical University, Tbilisi, Georgia*

(Presented by Academy Member Tengiz Beridze)

The goal of the present paper is the study of γ-irradiation (^{137}Cs) on conformation of DNA double helix in NaCl and $NaNO_3$ solutions using UV spectrometry and LI FRET (Laser induced fluorescence resonance energy transfer) spectroscopy. Effect of γ-irradiation dose of 26.4 krad on thymus DNA in 0.01M NaCl and $NaNO_3$ solutions (pH=6) has been studied. DNA concentration was $5.6 \cdot 10^{-4}$ M (P). The study was carried out by UV spectrometry and LI FRET spectroscopy. Total amount of primary products of water radiolysis at neutral *pH* after 26.4 krad irradiation was nearly ~0.6 on a single DNA nucleotide. Under irradiation of DNA in NaCl solution the efficiency of energy transfer, E_{ET}, from AO to EB intercalated in double helix is changed from 0.88 to 0.92. Registration of DNA UV absorption spectra at λ≅260 nm demonstrated distraction of 9% of bases; single strand scissions in DNA-NaCl is one distraction per 100 DNA nucleotides. When ethanol (0.1 M) is present irradiation practically does not distract nucleus bases but causes E_{ET} change from 0.82 to 0.89. At DNA irradiation in $NaNO_3$ solution the bases are not distracted but the irradiation induces E_{ET} change from 0.65 to 0.78. The presence of ethanol also does not distract bases and E_{ET} value is 0.66. It is shown that LI FRET spectroscopy applied with UV spectrometry is quite a sensitive method to detect structural defects (stress) of DNA double helix. *© 2022 Bull. Georg. Natl. Acad. Sci.*

DNA, FRET, γ-Irradiation, ionic strength

It is difficult to overestimate the value of both ionizing (X-ray and gamma radiation) and nonionizing (ultraviolet, visible and near infrared regions of the spectrum) electromagnetic radiation in medicine. Ionizing radiation damages all the molecules in a living cell but the damages in the DNA are most fatal. The most frequent types of radiation-induced DNA damages are: single- and double strand breaks, inter- and intrastrand cross-links, destruction, modification and release of nucleobases, local breakage of hydrogen bonds [1-4]. The radiation can affect DNA directly resulted from the direct interaction of radiation with the biomolecule, and also resulted from the interaction of DNA with

chemical products of water radiolysis [5-7]. DNA conformation, its ionic surrounding, water content, temperature, influence the radiation effect on the DNA. Radiation-induced DNA damages decrease at the rise of ionic strength and ethanol concentration in the solution [1,8-10]. In addition, of considerable interest is the use of non-equilibrium atmospheric pressure cold plasma in medicine.

In 2016 we developed the nanoscale method [11] of registration in real time of laser induced fluorescent resonance of energy transfer (FRET) between donor acridine orange (AO) and ethidium bromide (EB) acceptor intercalated in DNA macromolecule for the quantitative analysis of DNA and the analysis of DNA double helix quality after stress. It is shown that ions Cu(II), Cu(I), Ag(I) and AgNPs, and the effect of heating decrease the concentration of undamaged areas of DNA double helix, i.e. the sites able to intercalate dye molecules such as AO and EB.

FRET method allows to estimate the concentration of double helix areas with high quality stability applicable for intercalation in DNA after it was subjected to stress effect. It gives the opportunity to compare DNA-s of 1) different origin; 2) with various damage degrees; 3) being in various functional state.

Study of the effect of argon glow discharge irradiation (700-1800nm) on the conformation of DNA macromolecules also has been studied [12].

The goal of the present work is the study of gamma irradiation (^{137}Cs) on conformation of DNA double helix in NaCl and $NaNO_3$ solutions using UV spectrophotometry and laser induced FRET spectroscopy.

Materials and Methods

Materials. DNA in our tests, we used the calf thymus DNA (40% GC), Sigma-Aldrich (GPC JSC Tbilisi, Georgia). The concentration of nucleic acids was determined by UV absorption using molar extinction coefficients (ε=6600 M^{-1} cm^{-1} at λ=260 nm). The double helix structure of the polymers was proved by their hyperchromicity (>30%) and their typical thermal denaturation transition (measured in 0.01 M $NaNO_3$, pH≅6.0). pH was checked by a pH meter HANNA Instruments pH213 (Woonsocket, USA).

Acridine orange (AO) was purchased from Sigma-Aldrich (GPC JSC Tbilisi, Georgia). The concentration of the dye was determined colorimetrically at the isobestic point of the monomer-dimer system (λ=470 nm) using the molar extinction coefficients (ε=43 300 M^{-1} cm^{-1}). Ethidium bromide (EB) was also purchased from Sigma-Aldrich (GPC JSC Tbilisi, Georgia). The concentration of the dye was determined colorimetrically (ε=5600 M^{-1} cm^{-1} at λ=480 nm).

Instrumentations. Absorption spectra of DNA complexes with intercalators AO and EB were registered in real time using charge-coupled device (CCD) spectrometer AvaSpec ULS 2048-USB2. It should be underlined that registration of fluorescence spectra excited by laser irradiation is necessary to carry out in real time as, at such, excitation of intercalators, AO in particular, its fast photo-oxidation takes place.

Diode laser SDL-475-100T (Shanghai Dream Lasers Technology Co., Ltd., Shanghai, China) was used for irradiation and excitation (λ=457 nm with optical beam cross section 2 mm, and P=50 mW) of laser-induced fluorescence spectra.

The study of gamma radiation exposure of DNA samples was carried out on – Radiation tool – "Gamma-kapsula". Source of radioisotope – ^{137}Cs, dose rate of 1.1 Gy/min. Plastic irradiation chamber #3 (10·5 sm). Temperature during the irradiation in the chamber - 20±5°C.

DNA samples in a solution of 0.01 M NaCl and 0.01 M $NaNO_3$ in the absence and presence of 0.1 M ethanol in a plastic test tube with a volume of 1.5 ml were irradiated for 4 hours. The radiation dose was 26.4 krad. The DNA concentration was $5.6 \cdot 10^{-4}$ per phosphate.

The UV absorption spectra of DNA were recorded in 2mm quartz cuvettes, the DNA

concentration was $5.6 \cdot 10^{-4}$ M per phosphate in solutions of 0.01 M NaCl and 0.01 M $NaNO_3$. The fluorescence spectra of AO in the complex with DNA and FRET were also recorded in solutions of 0.01 M NaCl and 0.01 M $NaNO_3$ in 1 cm quartz cuvettes. The concentration of DNA was $2.8 \cdot 10^{-4}$M for per phosphate, the concentrations of the intercalators AO and EB were $0.7 \cdot 10^{-5}$ M.

Results and Discussion

Before reviewing the effect of gamma radiation on a DNA molecule, we want to pay special attention to the strange behavior of DNA macromolecules found by us, which are found in solutions of NaCl and $NaNO_3$.

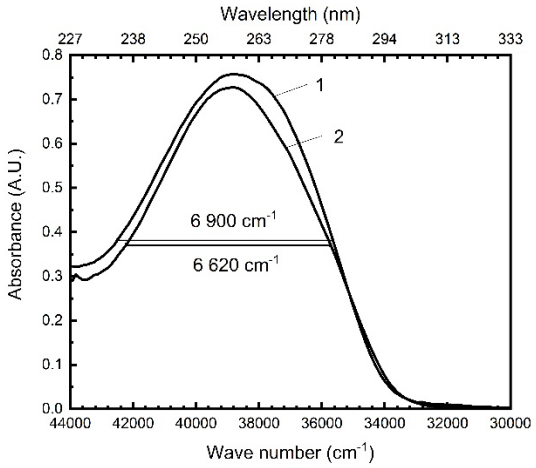

Fig. 1. UV absorption spectra of DNA in solutions of 0.01 M NaCl (1) and 0.01 M $NaNO_3$ (2).

Fig. 1 shows the UV absorption spectra of DNA in NaCl and $NaNO_3$ solutions in wave numbers. It can be seen that the absorption spectrum of DNA in a NaCl solution is shifted to longer wavelengths by 90 cm^{-1} and has a band broadening at half maximum by 240 cm^{-1}. We should note that the bathochromic shift of the DNA spectra from 6-700 cm^{-1} in the UV region is observed when DNA interacts with hydronium ions and transition metal ions, as well as when hydronium ions influences on UV absorption spectra of Guanosine and Cytidine G + C (pH 7) and G (pH 1) + C (pH 2) [13].

Besides, a similar shift from 200 to 700 cm^{-1} is observed during the conformational transition of DNA from the B form to the A, C and Z forms, as well as during the interaction of DNA with polyethylene glycol (Psi form of DNA), and ions of Cu (I), Ag (I), (inter cross link in DNA) [14] (see Fig. 2). We interpreted this bathochromic shift as double proton transfer (DPT) in GC pairs of DNA (see Fig. 2).

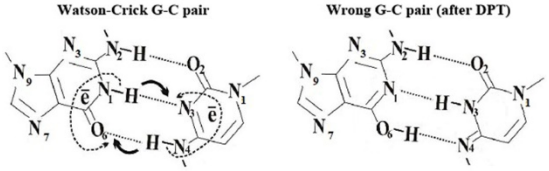

Fig. 2. Double proton transfer (DPT) in GC pairs of DNA.

The bathochromic shift of the absorption band in NaCl solution and the broadening of the band by 240 cm^{-1} give us reason to believe that in DNA in the above solution, as compared to DNA in $NaNO_3$, GC pairs are more in an irregular shape due to DPT in them. In this regard, DNA in NaCl will interact with intercalators, in particular with AO, worse than DNA in $NaNO_3$, which is actually observed by us (see Fig. 3). Besides, the FRET spectrum changes. In NaCl, the energy transfer efficiency E_{et} is 0.88, while in $NaNO_3 E_{et} = 0.67$.

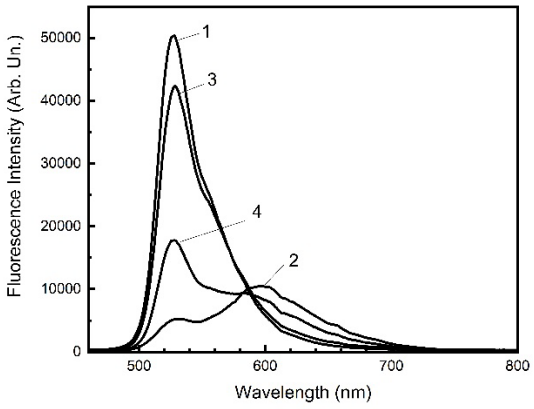

Fig. 3. Fluorescence spectra of AO - DNA complexes in solutions of 0.01 M NaCl (1) and 0.01 M $NaNO_3$ (3). The same figure shows the FRET spectra of AO-Ethidium bromide-DNA complexes in solutions of 0.01 M NaCl (2) and 0.01 M $NaNO_3$ (4). AO fluorescence was excited at lambda = 457 nm and recorded in 1 cm quartz cuvette.

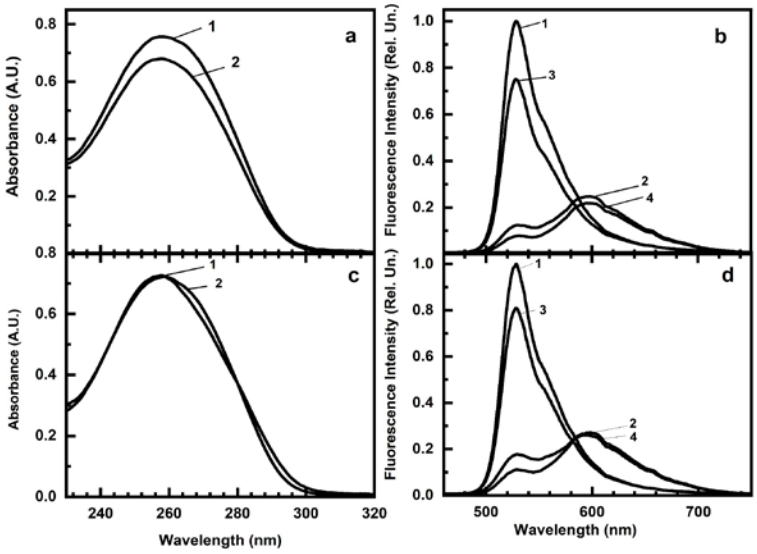

Fig. 4. UV absorption spectra of unirradiated DNA (1) and irradiated with gamma radiation in solutions of 0.01 M NaCl (a), and of 0.1 M ethanol and 0.01 M NaCl (c). Fluorescence spectra of AO-DNA unirradiated (1), and irradiated with gamma radiation (3), FRET spectra of AO-ethidium bromide-DNA complexes with unirradiated (2) and irradiated with gamma radiation (4) DNA in solutions of 0.01 M NaCl (b), and of 0.1 M ethanol and 0.01 M NaCl (d).

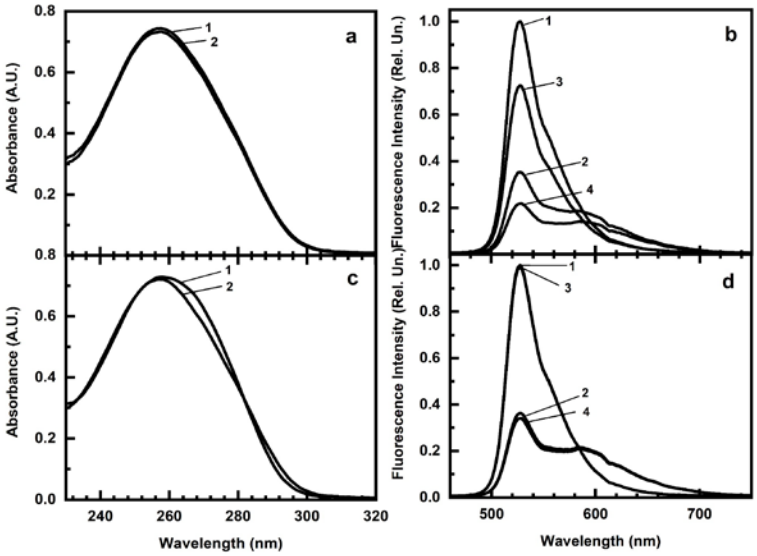

Fig. 5. UV absorption spectra of unirradiated DNA (1) and irradiated with gamma radiation of in solutions of 0.01 M $NaNO_3$ (a), and of 0.1 M ethanol and 0.01 M $NaNO_3$ (c). Fluorescence spectra of AO-DNA unirradiated (1), and irradiated with gamma radiation (3), FRET spectra of AO-ethidium bromide-DNA complexes with unirradiated (2) and irradiated with gamma radiation (4) DNA in solutions of 0.01M $NaNO_3$ (b), and of 0.1 M ethanol and 0.01 M $NaNO_3$ (d).

Effect of γ-irradiation dose of 26.4 krad on thymus DNA in 0.01 M NaCl and $NaNO_3$ solutions (pH=6) has been studied. DNA concentration was $5.6 \cdot 10^{-4}$ M (P). The study was carried out by UV spectrometry and laser induced FRET spectroscopy.

Under irradiation of DNA in NaCl solution the efficiency of energy transfer, E_{ET}, from AO to EB intercalated in double helix is changed from 0.88 to 0.92 (see Fig. 4). Registration of DNA UV absorption spectra at λ≅260 nm demonstrated distraction of 9% of bases; single strand scissions in DNA-NaCl is one distraction per 100 DNA nucleotides. This datum is in good agreement with the literature, in particular, after γ-irradiation of calf thymus DNA

(dose 26.4 kilorad), 8% of the double helix strands of DNA is destroyed [15] (radiation chemical yield G = 1.7). And in our case the demolition efficiency is 9%. When ethanol (0.1 M) is present irradiation practically does not distract nucleus bases but causes E_{ET} change from 0.82 to 0.89.

At DNA irradiation in $NaNO_3$ solution the bases are not distracted but the irradiation induces E_{ET} change from 0.65 to 0.78 (see Fig. 5). The presence of ethanol also does not distract bases and E_{ET} value is 0.66.

Ethanol strongly absorbs OH radicals due to addition reaction, it preserves DNA from nucleobase distraction. Besides, NO^-_3 anions neutralizing hydrated electrons e^-_{aq} preserve DNA from other defects such as single strand scissions, etc.

It should be noted that the effect of argon glow discharge irradiation (700-1800 nm) on the conformation of DNA molecules induces change of E_{ET} from 0.84 to 0.91 [12].

It is shown that Laser Induced FRET spectroscopy applied with UV spectrometry is quite a sensitive method to detect structural defects (stress) of DNA double helix.

Table 1. Radiation-chemical yields G [16] of the primary products of radiolysis of water per 100 eV of absorbed energy of gamma radiation (26.4 krad) and Q – the amount of radiolysis products per 1 DNA nucleotide

	e^-_{aq}	H^+	OH^-	OH	H_2O_2	H_2	H
G	2.6	2.8	0.1	2.6	0.7	0.45	0.5
Q	0.125	0.14	0.005	0.125	0.02	0.07	0.08

Table 1 shows the radiation-chemical yields G of the primary products of water radiolysis at neutral pH after gamma radiation (^{137}Cs) with a dose of 26.4 krad per 1 ml of DNA solution with a concentration of $5.6 \cdot 10^{-4}$ M (for phosphorus) and Q – the amount of products radiolysis per DNA nucleotide. The total amount of products G is ~0.6/1 DNA nucleotide.

Table 2. Rate constants for the reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals with DNA, Na^+, Cl^-, NO_3^-, H_3O^+ and Ethanol

Reactant	k (mol^{-1} s^{-1})		
	e^-_{aq}	H	OH
DNA	$5.25 \cdot 10^8$ [15]	$7.32 \cdot 10^7$[15]	$1.98 \cdot 10^9$[15]
Na^+	$<10^5$[17]	–	–
Cl^-	$<10^5$ [17]	–	$<10^3$[17]
NO_3^-	$1.1 \cdot 10^{10}$ [17]	$9.3 \cdot 10^6$[17]	$<5x10^5$[17]
H_3O^+	$2.36 \cdot 10^{10}$[17]	$2.6 \cdot 10^3$[17]	–
Ethanol	$<10^5$[17]	$1.6 \cdot 10^7$ [17]	$1.1 \cdot 10^9$[17]

Table 2 shows specific bimolecular rate constants for the reactions of hydrated electrons, hydrogen atoms and Hydroxyl radicals with DNA, ethanol and ions Na^+, Cl^-, NO_3^- and H_3O^+at neutral pH.

Table 3. E_{ET}[a] values from AO (donor) to EB (acceptor) intercalated in DNA and the distance R_b between them

Stress factor for DNA	E_{ET}	R(nm)
DNA-NaCl	0.88	2.28
DNA-NaCl –γ-irradiated	0.92	2.04
DNA-NaCl- ethanol	0.82	2.57
DNA-NaCl- ethanol –γ-irradiated	0.89	2.22
DNA-$NaNO_3$	0.65	3.21
DNA-$NaNO_3$–γ-irradiated	0.78	2.73
DNA-$NaNO_3$- ethanol	0.65	3.21
DNA-$NaNO_3$- ethanol –γ-irradiated	0.65	3.21
DNA-NaCl	0.84	2.48
DNA-NaCl-NIR-irradiated	0.91	2.10

[a] FRET from donor AO to acceptor EB.

[b] Relative concentration of DNA double helix areas applicable for AO and EB intercalation, where $R^0_{\text{AO-EB}}$ is the distance between AO and EB at initial DNA concentrations, $R^{st}_{\text{AO-EB}}$ is the distance between AO and EB after stress.

$R = R_0 \frac{1}{\sqrt[4]{\frac{1}{1-E_{ET}}-1}}$, where R_0=3.75nm

Table 3 presents the values of the efficiency of energy transfer E_{ET} = 1- q_d/q_{0d} from AO (donor) to EB (acceptor) intercalated in DNA and the distance

R between them before and after stress. The concentration of DNA was $2.8 \cdot 10^{-4}$ M (P), the concentrations of AO and EB were $0.7 \cdot 10^{-5}$ M. Therefore, the concentration of AO and EB per DNA nucleotide was 0.05, which corresponds to 1 intercalator per 10 base pairs. At the end of the table for comparison, we provide data on the influence of argon glow discharge irradiation (near-infrared irradiation – NIR) on the conformation of DNA molecules [12]. As already noted, the largest change in E_{ET} is observed when comparing DNA in solutions of NaCl and $NaNO_3$ and the reason for this is double proton transfer.

Comparative analysis of UV absorption spectroscopy of DNA and FRET shows that, except for the eighth case, the FRET method reliably responds to stress. As for UV spectroscopy, only in one case, namely gamma-irradiation of DNA in a NaCl solution, UV spectroscopy reveals a decrease in absorption by 9%, which is associated with the destruction of DNA nitrogen bases. Analysis of the data shown in Fig. 4 and Table 3 gives us reason to conclude that laser-induced spectroscopy FRET is a sensitive method for detecting structural defects of the DNA double helix, in particular, such as changes in the shape of the helix (BC), depurinization and single-strand breaks. It should be noted that UV absorption spectroscopy of DNA easily detects double helix defects of the DPP type. When comparing the absorption spectra of DNA in NaCl and $NaNO_3$ solutions, a shift of the absorption band by 90 cm^{-1} and a broadening of the absorption band of DNA in NaCl by 240 cm^{-1} are observed in comparison with the spectrum of DNA in $NaNO_3$.

Conclusions

The interactions of ligands, including intercalators, with DNA depend on the hydration capacity of the double helix, which in turn depends on the conformational forms of the helix: B, C, A, Z, and psi. These forms depend on the dielectric capacity of the solvent, the ions that create the solution and their ability to hydrate positively or negatively, and finally ionic strength.

Analysis of the data presented in the work gives grounds to conclude that laser-induced spectroscopy FRET, together with UV spectrometry, is a sensitive method for detecting structural defects in the DNA double helix, in particular, such as double proton transfer, spiral shape change, depurinization and single-strand breaks.

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ბიოფიზიკა

γ-დასხივების ეფექტი დნმ-ზე და ორმაგი სპირალის ხარისხის ანალიზი ლაზერით ინდუცირებული ფლუორესცენციის რეზონანსული ენერგიის გადატანის (FRET) მეთოდის გამოყენებით

ვ. ბრეგაძე*, ზ. მელიქიშვილი**, თ. გიორგაძე*, შ. გოგიჩაიშვილი*, მ. გოგებაშვილი*, ნ. ივანიშვილი*, ი. ხუციშვილი*

*ივანე ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი, ანდრონიკაშვილის ფიზიკის ინსტიტუტი, თბილისი საქართველო
**საქართველოს ტექნიკური უნივერსიტეტი, ვლადიმერ ჭავჭანიძის სახ. კიბერნეტიკის ინსტიტუტი, თბილისი საქართველო

(წარმოდგენილია აკადემიის წევრის თ. ბერიძის მიერ)

სამუშაოს მიზანი იყო ულტრაიისფერი სპექტრომეტრიის და ლაზერით ინდუცირებული ფლუორესცენციის რეზონანსული ენერგიის გადატანის (FRET) მეთოდის გამოყენებით NaCl და $NaNO_3$ ხსნარებში დნმ-ის კონფორმაციაზე γ-დასხივების ეფექტის შესწავლა. შესწავლილი იყო 26,4 კრად γ-დასხივების ეფექტი 0,01 მ NaCl და $NaNO_3$ ხსნარებში (*pH*=6) თიმუსის დნმ-ზე. დნმ-ის კონცენტრაცია იყო $5{,}6\cdot10^{-4}$ მ (ფოსფატზე). წყლის რადიოლიზის პროდუქტების სრული რაოდენობა ნეიტრალური *pH-ის* პირობებში, 26,4 კრად γ-დასხივების შემდეგ დნმ-ის ნუკლეოტიდზე არის დაახლოებით ~0,6. NaCl-ის ხსნარში დნმ-ის დასხივებისას მასში ინტერკალირებული აკრიდინ ნარინჯისფერიდან ეთიდიუმ ბრომიდზე ენერგიის გადაცემის ეფექტურობა, E_{ET}, იცვლება 0,88-დან 0,92-მდე. დნმ-ის ულტრაიისფერი შთანთქმის სპექტრი აჩვენებს 9% ფუძეების შეშფოთებას; დნმ- NaCl ხსნარში 100 ნუკლეოტიდზე ხდება ჯაჭვის ერთი წყვეტა. 0,1 მეთანოლის ხსნარში დასხივება პრაქტიკულად არ ზემოქმედებს ფუძეებზე, მაგრამ იწვევს E_{ET} -ის ცვლილებას 0,82-დან 0,89-მდე. დნმ-ის დასხივება $NaNO_3$ ხსნარში არ ვნებს ფუძეებს, მაგრამ დასხივება იწვევს E_{ET} -ის ცვლილებას 0,65-დან 0,78-მდე. ეთანოლის ხსნარში დასხივება ასევე არ ვნებს ფუძეებს და E_{ET} არის 0,66. ნაჩვენებია, რომ ლაზერით ინდუცირებული ფლუორესცენციის რეზონანსული ენერგიის გადატანის მეთოდის გამოყენება ულტრაიისფერ სპექტრომეტრიასთან ერთად მგრძნობიარე მეთოდია დნმ-ის ორმაგი სპირალის სტრუქტურული დეფექტების გამოვლენისთვის.

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