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Effect of Ni ions on the DNA spectral properties and photostability

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The optical absorption, fluorescence, phosphorescence in UV and visible spectral range, and effect of light irradiation on spectral properties of DNA with the presence of nickel ions are studied. The quantity of nickel ions varies from 1 ion per 500 base pairs of DNA to 2 ions per base pair. Three important features fixed: the shape of fluorescence and phosphorescence spectra of the DNA do not change in presence of Ni, but their intensity depends on the number of nickel ions in solution; phosphorescence intensity decreases more rapidly than fluorescence; the small decrease of DNA photodegradation rate in presence of Ni is observed. The average of triplet exciton path length evaluated from the dependence of phosphorescence/fluorescence intensity ratio on the relative concentration of nickel ions is 30–20 DNA pair sequence length. It was proposed that nickel ions interact in the outer side of DNA with the phosphate groups and do not penetrate intra macromolecular space.

Keywords: DNA, metal ions, photodegradation, fluorescence, phosphorescence.

Introduction

The interaction of vitally important DNA macromolecule with metal ions plays an important role in the conformation dynamic structure of DNA, its electronic and thermodynamic properties [1]. Partly in Ref. 2 the authors have shown that the thermostability of DNA to a great extent depends on type of metal ions that bind to DNA. On the other hand, the health effects of heavy metal toxicity in humans are observed [3]. So, the studies of the interaction of DNA macromolecule with metal ions are very significant. Especially important are knowledge concerning the sites of metal ions binding to DNA and related to these electronic processes in the macromolecule.

The effect of paramagnetic ions on DNA spectral properties was the subject of early works (see, e.g., [4]). Partly in Ref. 5, the results of investigations of the influence of the Mg^{2+} and Fe^{2+} ions on DNA phosphorescence were presented. It was found that these paramagnetic ions (or some centers connected with them) were the quenches of DNA phosphorescence. Moreover, the authors have found that triplet excitons were generated in the DNA macromolecule and the resulting triplet excitons path length is near the length of 20 DNA base pairs sequence along the macromolecule. Another important result was the fact that authors did not observe the increasing of the DNA phosphorescence intensity due to so-called “effect of heavy atom” (we mean here the possible phosphorescence intensity increasing under Fe^{2+}, Mn^{2+}ions incorporation into DNA macromolecule).

In this paper, we present the results on studying the effect of the Ni^{2+}ions on the low-temperature DNA luminescence and compare these results with the results that we have obtained in our previous works on studying the DNA–Co ions-containing system [4], DNA-Pt containing system and Berson–Isenberg results [5], obtained on the studying of DNA–Mn and DNA–Fe ions system.

Partly, we have attempted to answer on two questions:

(1) What is the path length of the mobile triplet excitations in DNA — paramagnetic ions system?

(2) Why “effect of the heavy atom” did not appear in DNA–Mn, DNA–Fe, DNA–Co, DNA–Ni systems under binding these ions to the DNA macromolecule?

The results of the optical absorption, fluorescence, phosphorescence in UV and visible spectral range, and effect of light irradiation on the spectral properties of DNA with different ratios of nickel are presented.

Experimental

Investigated materials: DNA, deoxyribonucleic acid from cattle spleen tissue was obtained from the Institute of Oncology of AMSU; nickel nitrate was obtained from the Chemical faculty of the Taras Shevchenko National University of Kyiv.

$Ni(NO_3)_2$ (Nickel(II) nitrate) was diluted in distilled water. Concentration $3.0 \cdot 10^{-3}$ M was used as stock, and further diluted. The concentration of the samples was much lower than the critical solubility, and therefore we consider that $Ni(NO_3)_2$ is completely dissociated. In water solution, it dissociates to Ni^{2+} ions, which interact with DNA macromolecules. NO_3^- groups can be seen in the absorption spectrum at the 200–240 nm range (see Fig. 1) and it does not affect on the DNA absorption spectra in the investigated area near 260 nm.

DNA was dissolved in pure distiller water to concentration $15 \cdot 10^{-4}$ $M_{base\ pairs}$, and this solution was used as a stock. This stock solution was diluted in 20 times to obtain working samples for further addition of solution of $NiNO_3$.

The concentration of DNA in different samples was identical — $7.5 \cdot 10^{-5}$ $M_{base\ pairs}$, the concentration of nickel ions varies from 1 nickel ion per 500 DNA base pairs to 2 nickel ions per 1 DNA base pair.

All investigated samples were prepared at room temperature $T = 24$ °C (297 K).

The same compounds which were also prepared at $T =$ = 80 °C (353 K) show the same experimental results.

The steady-state fluorescence and phosphorescence measurements were performed with Cary Eclipse spectrofluorometer (Varian, Austria) at $T = 77$ K, the liquid sample is frosted in Optistat DN nitrogen cryostat (Oxford Instruments, USA); optical absorption spectra were recorded on a Specord UV–VIS spectrophotometer (Carl Zeiss) at ambient temperature.

Photodegradation experiments were performed at ambient temperature, the liquid samples located in standard quartz cuvettes (light path = 10 mm) were irradiated by UV and visible light of Hg lamp DRT-1000 (1000 W) without additional optical filters. The light was focused on cuvettes by the quartz lens, which is transparent to UV light. One of the powerful lines in the Hg lamp emission spectrum (254 nm) falls near the maximum of the absorption spectrum of the DNA macromolecule.

Results and discussion

The absorbtion of DNA–$Ni(NO_3)_2$ *water solutions*

The absorption spectra of the pure DNA and DNA–$Ni(NO_3)_2$ water solutions of different Ni ions concentrations were shown in Fig. 1.

The absorption spectra of the DNA–$Ni(NO_3)_2$ solutions are similar to the spectrum of pure DNA. Changes in the range 220–240 nm are connected, to our opinion, with the increasing number of NO_3^- groups in solution. It does not affect on the DNA absorption spectra in the investigated area near 260 nm.

The shape of the spectra near the main band of DNA absorption 260 nm, and the wavelength of maximum in absorbtion do not depend on nickel ions quantity, so conclusion can be made that the nickel ions do not form

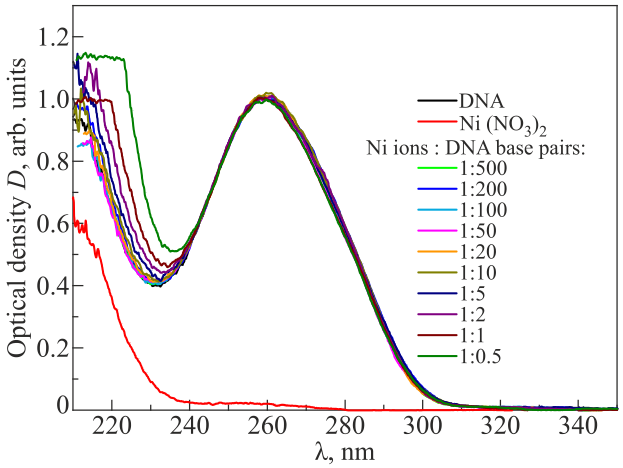


Fig. 1. (Color online) The absorption spectra at DNA, DNA–Ni water solutions, and $Ni(NO_3)_2$ water solution with the same concentration as in DNA–Ni (1:1). $T = 293$ K.

complexes with DNA bases in the ground state because namely DNA bases are responsible for light absorption in this spectral range.

The luminescence of DNA–$Ni(NO_3)_2$ *water solutions*

The pure nickel ions in solution did not manifest itself in fluorescence and phosphorescence. The NO_3 groups do not observe in fluorescence too. The shape of the fluorescence and phosphorescence spectra of DNA and DNA–Ni samples do not depend on nickel ions quantity. The fluorescence and phosphorescence spectra of these samples are near to the fluorescence and phosphorescence of pure DNA, but both have a lower intensity in comparison to pure DNA spectra.

From Fig. 2 it follows that DNA–Ni fluorescence and phosphorescence intensity both are decreasing with the increasing number of nickel ions in the sample. It should be noted that phosphorescence intensity decreased more rapidly than the fluorescence (see Fig. 3).

The shape of the fluorescence and phosphorescence spectra that are connected with C and G bases of DNA (fluorescence) [9] and AT traps (phosphorescence) [6, 9] emission do not change at ions doping. The changes of the intensity, to our opinion, can be explained by quench action of nickel ion or of some their complexes (like exciplexes, manifested very weak quantum yield of emission) with negative charged phosphate groups which are located on the periphery of the macromolecule.

With the increase number of nickel ions, the phosphorescence intensity decreases more rapidly than the fluorescence intensity; therefore the I_{phos}/I_{fluo} ratio is decreasing (Fig. 4).

Such influence of the metal ions on optical properties of DNA was observed in our previous work [4] for cobalt ions and in more early work of Berson and Isenberg for Mn and Fe ions [5]. We agree with Berson and Isenberg

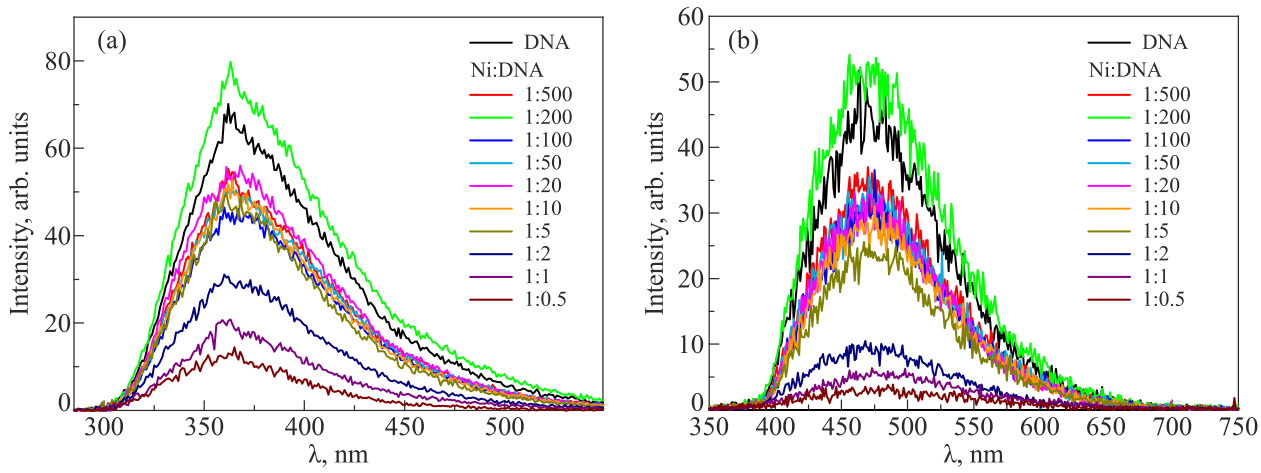


Fig. 2. (Color online) The fluorescence (a) and phosphorescence (b) of DNA spectra and DNA–Ni solutions with different quantities of nickel. Excitation was at 260 nm, $T = 77$ K.

that observed effects of DNA phosphorescence quenching by paramagnetic ions are connected mainly with energy transfer by triplet excitons from DNA bases to some triplet traps connected with these ions. The similar energy transfer way we assume in DNA–Ni samples (see Fig. 5).

Moreover, as it follows from Fig. 4 (for DNA–Ni) and Fig. 4 in Ref. 4 (for DNA–Co) the essential decrease of relative DNA phosphorescence intensity starts when the average distance between two neighbor quenching centers (points of positive metal ions building to DNA negative charged phosphore groups) is 30–20 base pair sequence length. Namely this value corresponds to an average value of triplet exciton path length. This value is close enough to obtained by Berson and Isenberg (20 base pair) and our

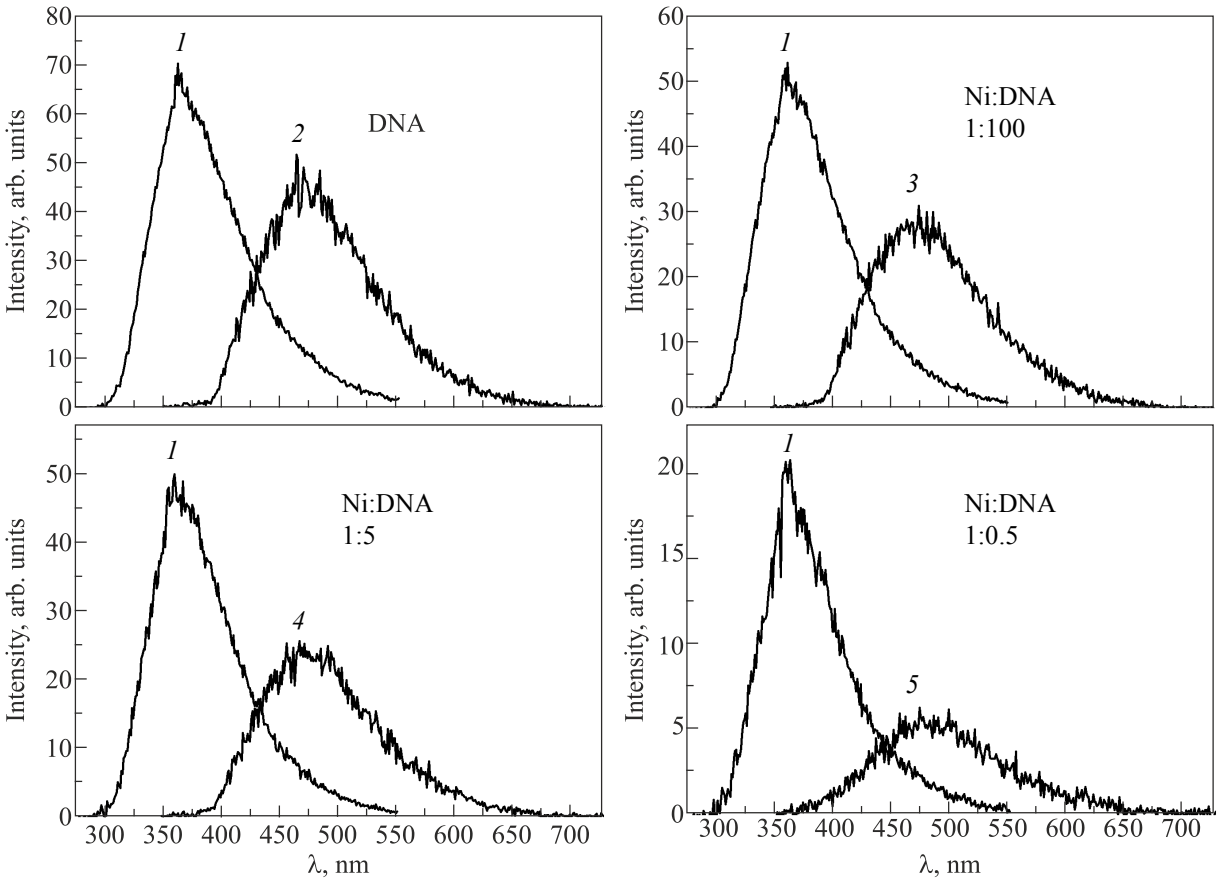


Fig. 3. The fluorescence (*1*) and phosphorescence (*2*)–(*5*) spectra of DNA and DNA–Ni-ion solutions with the different quantity of nickel. The spectra are shown at the same scale of fluorescence. Phosphorescence intensity multiplied ×20 at all spectra for clarity. Numbers 1:100, 1:5, 1:0.5 means Ni–DNA base pair ratio. $T = 77$ K, excitation 260 nm.

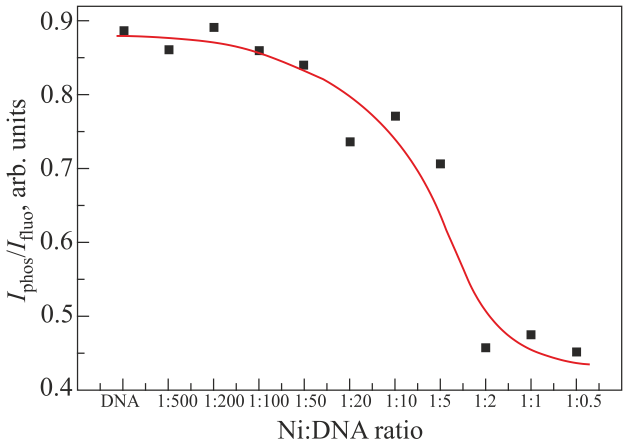
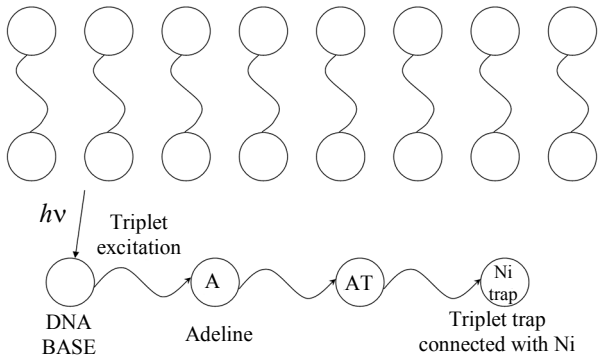


Fig. 4. The DNA phosphorescence/fluorescence intensity ratio dependence on the nickel ions quantity. The red curve was constructed using the paired point method.

previous works (16–20 base pair) [6–9] obtained by other methods. It should be emphasized that for RNA the average value of triplet excitons path length exceeds the length of 30 RNA bases [9, 13].

The second essential feature is that we did not observe the effect of "heavy atom" in the studied system — increasing of the DNA phosphorescence intensity inducing by the nickel or cobalt ions. The same peculiarities for the system of DNA–Mn, Fe ions were observed in Ref. 5. To our opinion, positive charged Ni, Co, Mn, Fe ions do not penetrate intra DNA (between base pairs) space but bind mainly to the negative charged phosphate groups on the periphery of the DNA macromolecule. Possibly, in this case wave functions of DNA basis and metal ions do not overlap enough for appearing of the "heavy atom" effect.

The fact of the similarity in fluorescence and phosphorescence spectra of DNA–nickel solution and pure native DNA proves that binding of the Ni^{2+}, as well as Co^{2+} ions to the DNA, does not affect essentially the positions of the



$$E_{T\ \text{A-base}} > E_{T\ \text{AT-trap}} > E_{T\ \text{Ni-trap}}$$

Fig. 5. The possible energy transfer way in the DNA–Ni system. The excitons generated in one of the DNA base are spreading along macromolecule to the centers that are low energy traps.

DNA bases singlet and triplet levels. These results also suggest in favor the fact that nickel and cobalt ions interact with the outer side of DNA with the phosphate groups. To additionally check it, the same samples of DNA–Ni compounds were prepared at $T = 80$ °C (353 K) and they show the same results: the presence of Ni ions do not affect to the shape of absorption and luminescence spectra, but changes in the intensity of DNA luminescence have occurred.

Study of DNA–Ni *photodegradation*

The absorption spectra of the DNA–Ni and the pure native DNA were investigated under irradiation of the samples of these compounds by UV and visible light of high intensity. Under irradiation, the photochemical process in DNA macromolecule occurs, which consequently leads to damage the DNA, and as a result, optical density is significantly decreased [9]. Meanwhile, the location and shape of the main band in absorption spectra of DNA and DNA–Ni do not change during irradiation (Fig. 6). Moreover, it easy to see that there is no significant difference between rates of DNA–Ni ions degradation and pure DNA. It is worth to stress that such type of results was obtained for

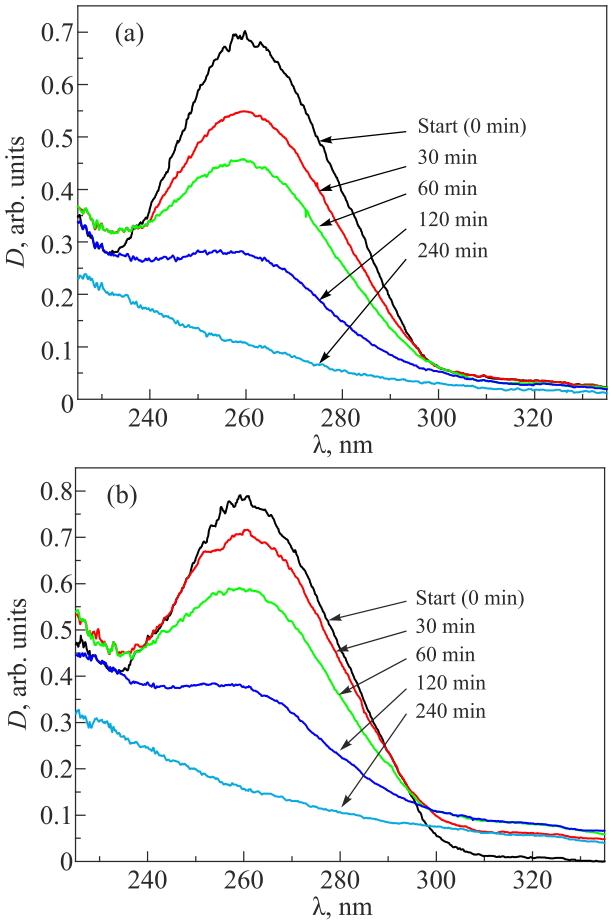


Fig. 6. (Color online) The dependence of optical density on the time of irradiation of the DNA (a) and DNA–Ni with ratio 1:5 (1 Ni ion per 5 DNA base pair), (b) under irradiation of ultraviolet and visible light of 1 kW Hg lamp.

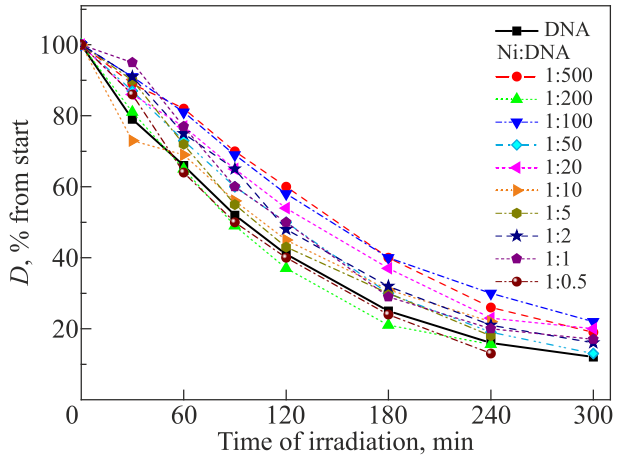


Fig. 7. (Color online) The time dependence of optical density D of the DNA absorption band 260 nm on the time of irradiation the DNA and DNA–Ni solution.

DNA–cobalt system too [4]. The similar results were obtained for all investigated DNA–Ni ratios. Comparing the results, some insignificant decrease of DNA degradation rate is observed in the DNA–Ni samples (Fig. 7).

It is noticeable that intensity decrease rate of the DNA sample is slightly faster, compared to DNA–Ni samples.

Comparing the degradation rates, the conclusion can be made that the influence of nickel ions on the DNA macromolecule leads to the small decrease of the DNA macromolecule photodegradation rate. After 180–240 minutes of irradiation, optical densities of DNA–Ni samples are 10–15 % higher than DNA. Unlike [10], where the influence of platinum was significantly more noticeable, the change of photodegradation rate of DNA with the presence of nickel is insignificant.

It is known that photochemical damage of organic molecules started from their excited triplet states [6, 11, 12]. The obtained spectral results prove that the population of excited triplet sites does not increase essentially in studied DNA–Ni (Co) systems compare with pure DNA. So there is no reason for increasing the rate of photochemical damage of DNA–Co system compared with pure DNA. To our opinion, this effect can be additional evidence that nickel and cobalt ions bind to DNA outside. This effect is contrary to the DNA–Pt sample, it is known that incorporation of Pt atoms inside DNA helix leads to increasing the intensity of DNA phosphorescence and significant decreasing the photostability of DNA–Pt complex compare to pure DNA. At the similar sets of experiments, the optical density of the DNA absorption band 260 nm in DNA–Pt solution decreases much more rapidly than in pure DNA [10].

Conclusions

Presence of Ni ions decreases the DNA fluorescence and phosphorescence intensity; meanwhile, it does not affect to absorption, fluorescence, and phosphorescence spectral band position and shape. It means that Ni ions do not affect the positions of the singlet and triplet levels of DNA bases but they are connected with traps for singlet and triplet excitons, spreading in DNA macromolecules.

The average of triplet excitons path length evaluated from the dependence of phosphorescence/fluorescence intensity ratio on the relative concentration of Ni ions is 30–20 DNA base pair sequence length. This value is close to one obtained earlier (Bergson's and Isenberg's result — near 20 DNA base pair sequence length, obtained for DNA–Fe, DNA –Mn ions system).

The presence of nickel (cobalt) ions does not affect on the DNA photostability under UV light irradiation; only weak increase of DNA degradation rate is observed.

To our opinion, the obtained results can be applied in ecology and nanomedicine for the development of new drugs for photodynamic therapy.

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Вплив іонів Ni на спектральні властивості ДНК та її фотостабільність

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Представлено результати досліджень оптичного поглинання, флюоресценції та фосфоресценції в УФ та видимому спектральному діапазоні, а також вплив опромінення на спектральні властивості ДНК у присутності іонів нікелю. Кількість іонів нікелю варіювалась від одного іону на 500 пар основ ДНК до двох іонів на одну пару основ ДНК. Зафіксовано три важливі особливості: форма спектру флюоресценції та фосфоресценції ДНК не змінюється в присутності іонів нікелю, але їх інтенсивність залежить від кількості металу в розчині; інтенсивність фосфоресценції спадає швидше, ніж інтенсивність флюоресценції, спостерігається незначне зменшення швидкості фотодеградації ДНК в присутності іонів нікелю. Із залежності співвідношення інтенсивностей фосфоресценції та флюоресценції від кількості іонів нікелю оцінено довжину середнього пробігу триплетного екситону, яка дорівнює приблизно довжині послідовності 20–30 пар основ ДНК. Зроблено припущення, що іони нікелю взаємодіють з зовнішніми структурами ДНК, які містять фосфатні групи, та не проникають всередину макромолекули.

Ключові слова: ДНК, іони металів, фотодеградація, флюоресценція, фосфоресценція.