Effect of Co Ions on the Spectral Properties and Photostability of DNA

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Summary: The results of the optical absorption, fluorescence, phosphorescence in UV and visible spectral range, and effect of light irradiation on spectral properties of cobalt ions of different ratios interacted with DNA are studied. Quantity of Co ions varies from 1 metal ion per 500 base pairs of DNA to 2 ions per one base pair. Three important features were fixed: the shape of fluorescence and phosphorescence spectra of the DNA do not changes in presence of Co ions, but their intensity depends on the quantity of cobalt ions in solution; phosphorescence intensity decreases more rapidly than fluorescence intensity; the small increase of DNA photodegradation rate in presence of Co is observed.

Keywords: cobalt; DNA, fluorescence; metal ions; photodegradation; UV-vis spectroscopy

Introduction

Specificity of biological reaction (metal – deoxyribonucleic acid (DNA)) allows, for the low concentrations of heavy metals, define and estimate toxicity and influence of these metals on the DNA macromolecule. The purpose of this work is to research the influence of cobalt metal ions on the spectral properties of DNA.

The studies of the physical aspects of interaction of Co ions with DNA are useful for understanding the low-level mechanism of their interaction. Optical methods of investigation have significant advantages over the other methods because they are non-destructive and can be used to study investigated materials in different states.

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 cobalt ions are studied.

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Experimental Part

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

Cobalt ions were obtained from $Co(NO_3)_2 \cdot 6H_2O$ – Cobalt(II) nitrate, which was diluted in distilled water. In water solution it dissociate to Co^+ ions, which interact with DNA macromolecules and to NO_3^-. NO_3^- groups can be seen in absorption spectrum at the 200–240 nm range (see Figure 1) and it do not affect on the DNA absorption spectra in the investigated area near 260 nm.

Concentration of DNA in different samples were identical – $7.5 \cdot 10^{-5} M_{(base\ pairs)}$, concentration of cobalt ions varies from 1 cobalt ion per 500 DNA base pairs to 2 cobalt ions per 1 DNA base pair.

All investigated samples were prepared at ambient temperature.

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

The steady state fluorescence and phosphorescence measurements were performed with Cary Eclipse spectrofluorometer at

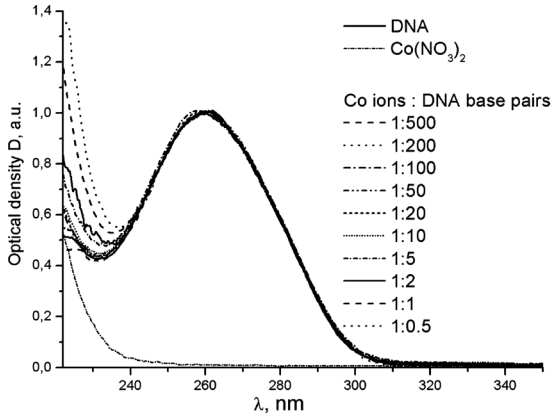

Figure 1.
The absorption spectra at T = 293 K of DNA, DNA–Co solutions, and Co solution with the same concentration as in DNA–Co 1:1.

T = 77K; optical absorption spectra were recorded on a Specord UV–VIS spectrophotometer at ambient temperature.

Photodegradation experiments were performed at ambient temperature, the samples in quartz cuvettes were irradiated by UV and visible light of Hg lamp DRT-1000 (1000W). One of the most powerful lines in the Hg lamp emission spectrum (254nm) falls near the maximum of the absorption spectrum of the DNA macromolecule.

Results and Discussion

The Nature of Absorbing Centers in DNA–Co

The absorption spectra of the pure DNA and DNA–Co solutions of different concentrations are shown in Figure 1.

The absorption spectra of the DNA–Co complexes are similar to the spectrum of pure DNA. Changes in range 220-240 nm are the consequence of the increasing number of NO_3^- groups in solution. It do 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 do not depends on cobalt ions quantity, so conclusion can be made that the cobalt ions do not bended into complexes with DNA bases.

The Luminescence of DNA–Co

The pure cobalt does not appear in fluo– and phosphorescence. The shape of the fluorescence and phosphorescence spectra of DNA and DNA–Co samples do not depends on cobalt quantity: the fluorescence and phosphorescence spectra of DNA–Co samples is near to the fluo– and phosphorescence of pure DNA, but both has a lower intensity in comparison to pure DNA.

In Figure 2 we can see that DNA–Co fluorescence and phosphorescence intensity both are decreasing with the increasing number of cobalt ions in a sample.

The shape of the fluorescence and phosphorescence spectra do not change, so we cannot say that DNA–cobalt complex is appear in exited state. The changes in intensity can be explained by the energy transfer from the DNA bases to cobalt.

With the increase number of cobalt ions, the phosphorescence intensity decrease more rapidly than the fluorescence intensity, therefore the I_{phos}/I_{fluo} ratio is decreasing (Figure 4).

To our opinion, such dramatic changes in luminescence spectra of DNA–Co solutions compared with the pure native DNA are connected with the influence of Co atoms.

A rapid decrease of intensity of the phosphorescence and decrease of I_{phos}/I_{fluo}

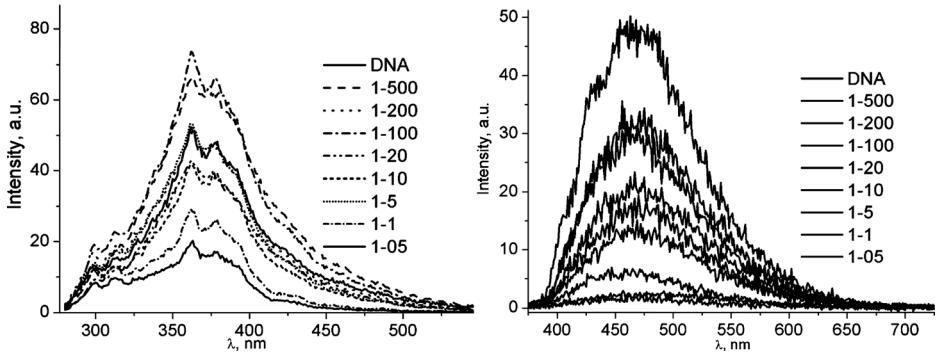

Figure 2.
The Fluorescence (left) and phosphorescence (right) spectra of DNA and DNA–Co solutions with different quantity of cobalt. Excitation 260nm, T = 77 K.

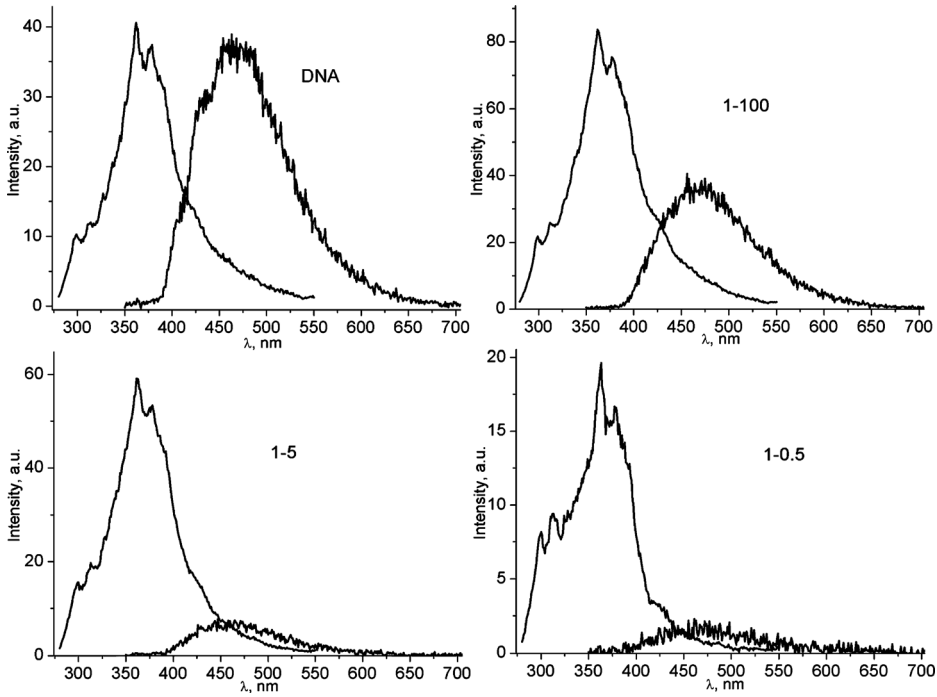

Figure 3.
The fluorescence (left peak) and phosphorescence (right peak) spectra of DNA and DNA–Co solutions with the different quantity of cobalt. The spectra are shown at the same scale of fluorescence. Phosphorescence intensity multiplied 20x at all spectra for clarity. Numbers 1–100, 1–5, 1–0.5 means cobalt/DNA base pair ratio. T = 77K, excitation 260nm.

ratio with the increasing amount of cobalt atoms, can be connected with a more effective triplet excitation energy transfer through DNA bases and it deactivation by cobalt ions.

Large bend on region near 1-20…1-10 of the I_{phos}/I_{fluo} ratio on Figure 4 can be a consequence of the length of triplet excitation migration path of 10-20 base pairs through DNA macromolecule. This result

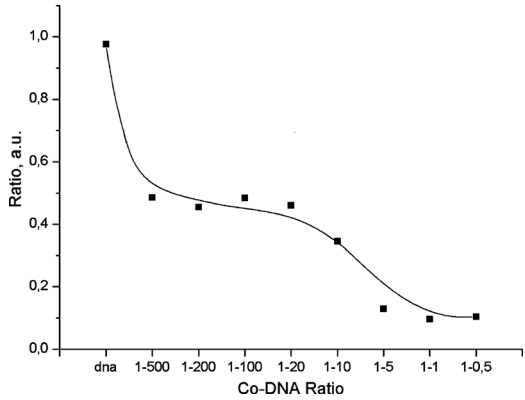

Figure 4.
The phosphorescence/fluorescence intensity ratio dependence of the cobalt ions quantity.

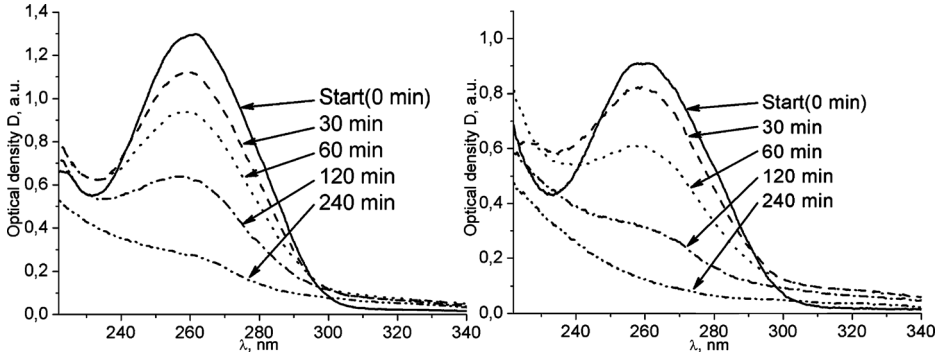

Figure 5.
The dependence of optical density on the time of irradiation of the DNA (left) and DNA–Co 1-5 (right) under irradiation of ultraviolet and visible light of 1 kW Hg lamp.

matches the known data that triplet excitation path length is near 16 DNA base pairs.[1]

The fact of the similarity in fluorescence and phosphorescence spectra between DNA–cobalt solution and pure native DNA proves that binding of Co atoms (or ions) to the DNA does not affect essentially the positions of the DNA bases singlet and triplet levels. These results correspond with the fact that cobalt ions interact with the outer side of DNA to the phosphate groups.[2–5] To additionally check it, the same samples of DNA–Co compounds were prepared at $T = 80^{\circ}C$ (353K) and they shows the same results – presence of Co ions do not affect to the shape of absorption and luminescence spectra, but changes in intensity of DNA luminescence are occurred.

Study of DNA–Co Photodegradation

The absorption spectra of the DNA–Co and the pure native DNA were investigated under irradiation the samples of these compounds by UV and visible light of high intensity. Under irradiation, photochemical process in DNA macromolecule occurs, which consequently lead to damage the DNA, and as a result, optical density is significantly decreased.[6] Meanwhile, the location and shape of the main band in

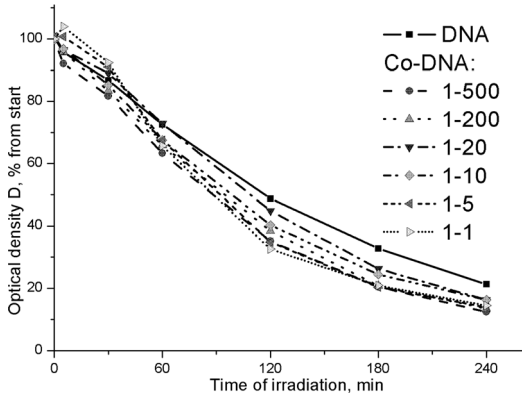

Figure 6.
The time dependence of optical density of the DNA absorption band 260nm on the time of irradiation the DNA and DNA–Co solution.

absorption spectra of DNA and DNA–Co do not change during irradiation (Figure 5).

The similar results were obtained for all the investigated DNA–cobalt ratios. This effect can be additional evidence that cobalt ions binding to DNA outside and do not effect essentially the positions of the singlet and triplet energy levels of DNA bases.

It is noticeable that intensity decrease rate of a DNA sample is slightly slower, compared to DNA–Co samples.

Comparing the degradation rates, conclusion can be made that the influence of cobalt ions on the DNA macromolecule leads to the small increase of the DNA macromolecule photodegradation rate. After 180-240 minutes of irradiation, optical densities of DNA–Co samples are 10-15% lower than DNA. Unlike,[7] where the influence of platinum was significantly more noticeable, value of the change of photodegradation rate of DNA with presence of cobalt is slight.

Conclusion

1. Presence of Co ions decreases the DNA fluorescence and phosphorescence intensity, meanwhile it does not affect to absorption spectra and to the positions of the singlet and triplet levels of DNA bases.
2. The phosphorescence/fluorescence intensity ratio is dependent on the cobalt ions quantity. This can be explained by more effective triplet excitation energy transfer, than a singlet, from DNA bases to the cobalt ions.
3. Presence of Co ions slightly decreases the DNA resistance to photodegradation of UV and visible light. It is shows up in more rapidly decrease of optical density of DNA–Co solution comparing to pure DNA under the same intensity of irradiation.

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