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SOME PECULIARITIES OF ELECTRONIC EXCITATION ENERGY STRUCTURE OF THE BIOLOGIC POLYNUCLEOTIDES AND PROCESSES OF TRIPLET EXCITATION TRAPPING

In the present paper some peculiarities of electronic excitation energy transfer in the DNA and RNA were examined and analyzed. The investigations of the absorption and phosphorescence spectra of the DNA and the model compounds showed that AT sequences are main triplet traps for the native DNA, and the nature of the DNA phosphorescence is mainly determined by these AT-sequences. Data obtained in our investigations prove the existence of the DNA self-protection mechanism against damage caused by UV-irradiation exists. So the hierarchy of the DNA bases electronic levels is not random and favors the electronic excitation energy transfer to photostable centers in the DNA (A-bases and AT-complexes). From the absorption and fluorescence spectra of the ribonucleotides the position of the first excited singlet (S_1) electronic energy levels of the RNA bases were evaluated (on the intersection of blue edge of fluorescence spectra curve and long-wave edge of absorption spectra). The positions of the first excited triplet (T_1) electronic energy levels of the RNA bases were evaluated using blue edges of the phosphorescence spectra of the ribonucleotides. It was shown that the triplet traps in the RNA are: adenosine groups and the centers of unknown nature.

Introduction

The biologic molecules and structures are the functional systems because they play determine role in living objects. Besides, some functional properties and mechanisms have to exist that favor to stability of biological molecular systems. We have done some attempt [1, 2] to understand, is there any mechanism of the DNA self protection against photoreactions or more exactly against reactions which are initiated by excitation of the DNA p-electron systems (for example, in radiation field). We believe the Nature have designed such mechanism in the DNA. Is (in this connection) the hierarchy of the DNA electronic singlet and triplet levels random? Or is there some logic in their displacement? We hope our paper gives some version of answer on this question.

We have decided to apply our experience and methods to study the electronic excitation energy transfer in the DNA and RNA. It was shown the thymidine-containing low-molecular compound essentially less photostable then the DNA. From the other hand, well-known papers of Gueron, Shulmann and Longworth [3–5] proved the triplet energy level of thymidine was the lowest among the DNA bases. It means the photophysical destroying rates of the DNA and thymidine low model compound have to be closed. But it was not the case. It was appeared namely the destroying rate of adenosine (contrary to other bases) model compound is more close to the DNA rate. So, we were forced to check the relative positions of adenosine and thymidine bases once again using low temperature phosphorescence measurements of these model compounds solutions. The result – the adenosine triplet level was the lowest [1].

Results and Discussion

Our investigations [1,2] showed the electronic absorption spectrum of the DNA and oligonucleotide d(CCCGGGTTTAAA) (specially designed and synthesized for the study of the DNA phosphorescence centers nature) is close to the additive sum of the spectra of the corresponding nucleotides (fig. 1, a) that is typical for nonconjugated p-electron-containing polymers; thus the nucleotides are practically independent absorbing centers in the DNA macromolecule and electronic processes in it start from initially excited the DNA bases, similarly as it takes place for the synthetic aromatic-containing nonconjugated macromolecules [6–8]. Contrary to absorption the phosphorescence measurements of the DNA, double-stranded polymer poly$(dAdT)_2$, the short synthetic DNA-fragments – d(CCCGGGTTTAAA), trimer d(ATC) (4) and dimer d(AT) – showed the triplet excitations localized in the DNA macromolecule in the traps associated with AT-sequences and namely these traps are sources of the DNA phosphorescence (fig. 1, b) [1, 2]. Besides, it was shown

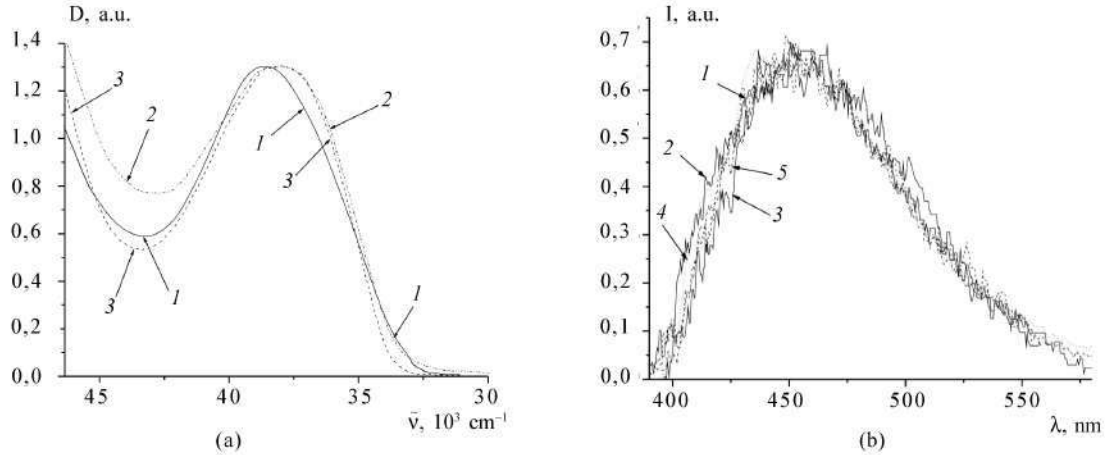

Fig. 1. The absorption spectra (a) of the total chicken DNA (1), d(CCCGGGTTTAAA) (2) and mixture (equimolar concentrations) of model compounds dAMP, dTMP, dGMP, dCMP (3); the phosphorescence spectra (b) of: poly(dAdT)$_2$ (1), d(CCCGGGTTTAAA) (2), DNA (3), d(AT) (4) and d(ATC) (5). Excitation 300 nm. Water solutions, C = 10^{-4}M

these DNA-sites were photochemically stable as well as adenosine groups. All these data proved the existence of the DNA self-protection mechanism.

The majority of the experimental investigations carried out by Gueron, Shulmann and Longworth [3–5] have been made using ribonucleotides that are the closest analogues of the RNA elementary links but not the DNA elementary links. In the most cases they tried to describe the nature of the DNA luminescent centers using ribonucleotides instead deoxyribonucleotides that is not correctly. In this connection the aim of our ongoing investigations is the comparison of the energy levels hierarchy of the DNA with the RNA and the study of the RNA luminescent centers nature comparing with the DNA. The absorption (at T = 300 K) and luminescence (at T = 4,2 K and 77 K) spectra of the RNA and ribonucleotides were investigated. It is worth to note the absorption spectra of the nucleic acids and nucleotides at 77 K [5] are generally similar to those observed at room temperature. From the absorption and fluorescence spectra (fig. 2, a–e) the position of the first excited singlet (S_1) electronic energy levels (fig. 3, a) of the RNA bases (rAMP, rGMP, rCMP, rUMP, rIMP) were evaluated (on the intersection of blue edge of fluorescence spectra curve and long-wave edge of absorption spectra). No fluorescence and no phosphorescence of rIMP were observed at T = 77 K that is why the energy levels of rIMP were presented only at T = 4,2 K.

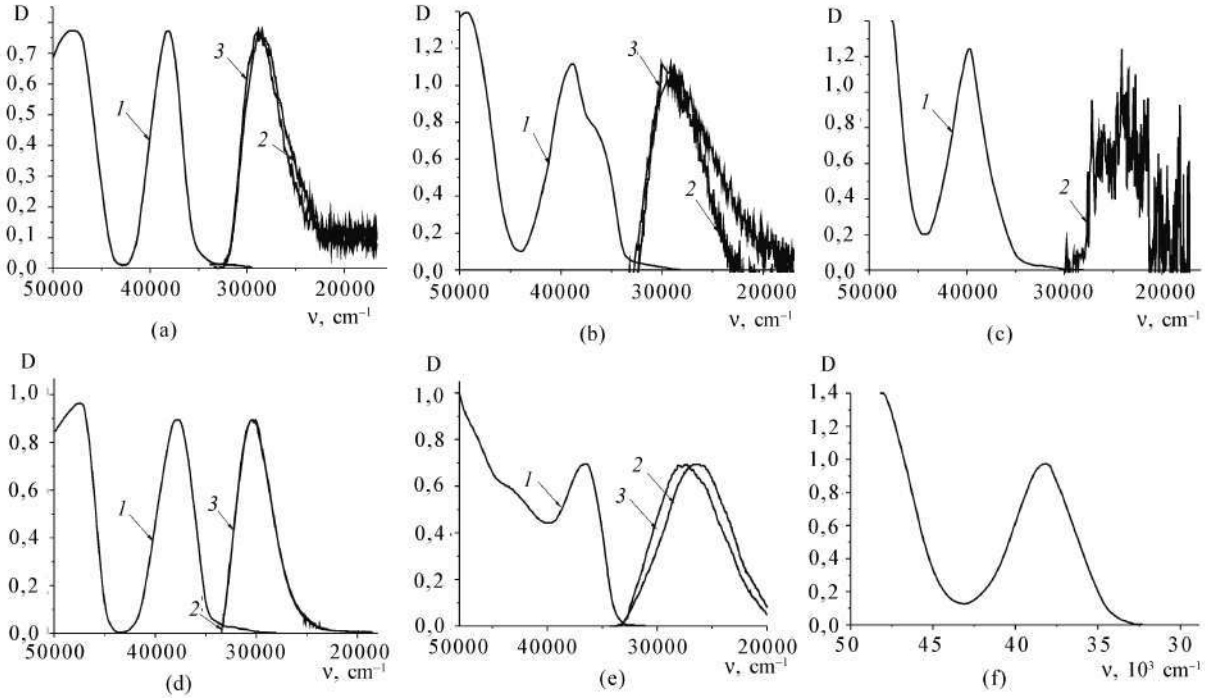

Fig. 2. The absorption spectra at 300K (*1*) and fluorescence spectra at 77K (*2*), at 4.2K (*3*) (excitation 260 nm) of: rAMP (a), rGMP (b), rIMP (c), rUMP (d), rCMP (e). The absorption spectra of RNA at 300K (f). Water solutions, C = 10^{-4} M

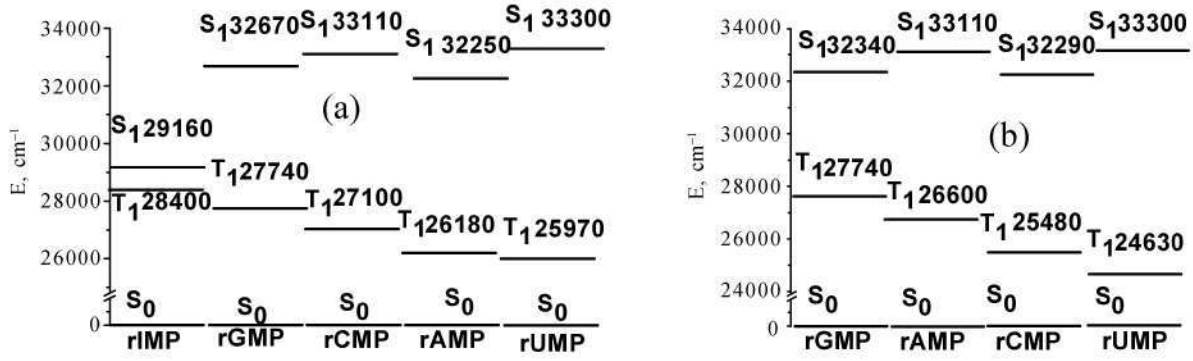

Fig. 3. The position of singlet and triplet energy levels of the RNA bases at $T = 4{,}2$ K (a) and $T = 77$ K (b)

The positions of the first excited triplet (T_1) electronic energy levels (fig. 3, b) of the RNA bases were evaluated using blue edges of the phosphorescence spectra (fig. 4, a–e) of the ribonucleotides (rAMP, rGMF, rIMP, rCMP, rUMP).

The comparison of the phosphorescence spectra of the RNA and the ribonucleotides (rAMP, rGMP, rIMP, rCMP, rUMP) shows that here are two type of triplet excitations traps: (1) adenosine groups and (2) the centers of unknown nature that manifested structureless long-wave phosphorescence (fig. 4, f). The results obtained on the RNA were compared with the results obtained on the DNA. This phosphorescence spectrum on the RNA do not coincide with the DNA phosphorescence spectrum. Thus, the different spectral properties and different photophysical processes take place in the RNA and DNA macromolecules in spite of the similarity in the chemical structure.

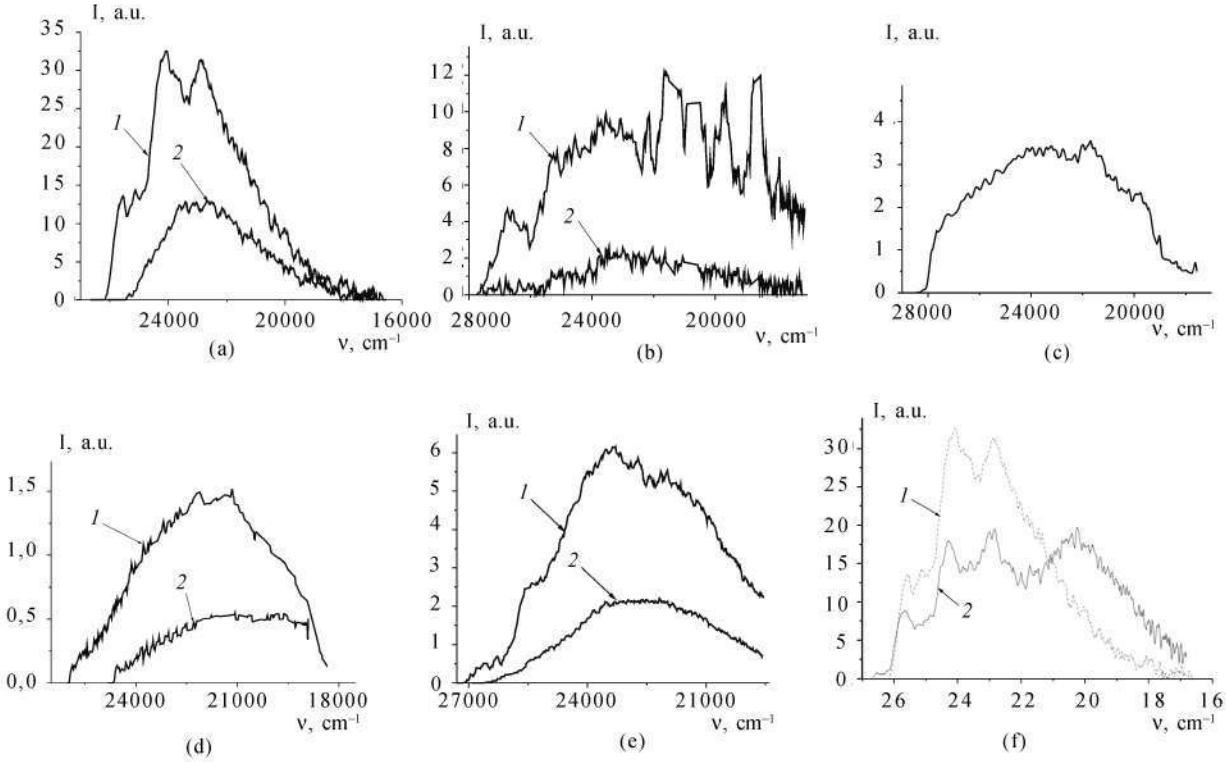

Fig. 4. The phosphorescence spectra at T = 4.2K (1) and T = 77K (2) of: rAMP (a), rGMP (b), rIMP (c), rUMP (d), rCMP (e); the phosphorescence spectra (f) of rAMP (1) and the total chicken RNA (2). Excitation 260 nm. Water solutions, $C = 10^{-4}$M

Conclusions

It is was shown by the comparative studies of the DNA and the model compounds (d(CCCGGGTTTAAA), d(AT) and d(ATC)) that AT sequences are main triplet traps for the native DNA, and the nature of the DNA phosphorescence is mainly determined by these AT-sequences [1,2]. The system of the RNA energy levels slightly differs comparing with the DNA system. To the other hand, the conditions for triplet electronic excitation migration, their capturing by traps are rather different contrary to the DNA that manifests the phospho-

rescence of the traps associated with AT-sequences. The RNA phosphorescence was emitted by adenosine bases and traps of unknown nature that manifested structureless long-wave phosphorescence.

Acknowledgement

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ДЕЯКІ ОСОБЛИВОСТІ ПЕРЕНЕСЕННЯ ЕНЕРГІЇ ЕЛЕКТРОННОГО ЗБУДЖЕННЯ В БІОЛОГІЧНИХ ПОЛІНУКЛЕОТИДАХ ТА ПРОЦЕСИ ЗАХОПЛЕННЯ ТРИПЛЕТНИХ ЗБУДЖЕНЬ

У даній статті розглянуто та проаналізовано деякі особливості перенесення енергії електронного збудження в ДНК та РНК. Дослідження спектрів поглинання та фосфоресценції ДНК та модельних сполук засвідчило, що саме АТ послідовності є основними пастками для триплетних збуджень в природні ДНК, а також саме вони визначають природу її фосфоресценції. Отримані дані підтверджують, що ієрархія електронних рівнів базових груп ДНК не лише не є випадковою, а й сприяє перенесенню енергії електронного збудження до фотостабільних центрів у ДНК, що в свою чергу забезпечує механізм самозахисту ДНК щодо згубної дії УФ випромінювання. Із спектрів поглинання та флюоресценції РНК і рибонуклеотидів визначено положення перших збуджених синглетних рівнів базових груп РНК (на перетині короткохвильового краю спектра флюоресценції та довгохвильового краю спектра поглинання). Положення перших збуджених триплетних рівнів було визначено використанням положення короткохвильового краю спектрів фосфоресценції. Показано, що основними пастками для триплетних збуджень в РНК є аденозинові групи та центри невідомої природи.