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THE SPECTRAL PROPERTIES OF THE TELOMERE FRAGMENTS

The optical absorption and phosphorescence (at low temperatures) of a telomere fragment (oligonucleotide d(AGGGTTAGGGTTAGGGTTAGGG)) and (for comparing) the DNA macromolecule are investigated under various excitation wavelengths (240–300 nm). Two types of d(AGGGTTAGGGTTAGGGTTAGGG) samples were studied: (1) the intact sample, and (2) the sample heated to 90°C and studied immediately after heating. It is concluded that the main traps of triplet electronic excitations in both these samples of d(AGGGTTAGGGTTAGGGTTAGGG), as well as DNA, are the complexes formed by neighbor adenine (A) and thymine (T) chromophores from the same strand (not from complementary chromophores of the different strands).

Keywords: optical absorption, phosphorescence, adenine chromophore, thymine chromophore, oligonucleotide d(AGGGTTAGGGTTAGGGTTAGGG).

1. Introduction

The deoxyribonucleic acid (DNA) is vitally important for any living objects. It plays the important role in the storage of genetic information. Telomere is the end part of the DNA macromolecule containing a large number of repeated sequences of 6 nucleotides – TTAGGG that are responsible for the DNA protection and reproduction (in addition, the AT-triplet excitation traps play the role of the DNA self-protectors too [1–3]). Without the telomere, the loss of an important genetic information takes place, which is resulted in whole chromosome rearrangements or apoptosis [4]. The telomere presence time in DNA determines mainly the number of cell divisions. The latter is the possible key to the solution of the cancer problem and the duration of the life.

Up to now, the studies of the spectral properties of telomers were carried out at ambient (or higher) temperatures and connected mainly with the spectral manifestation of G-G-quadruplex in it (see, for example, [5, 6]). There are no any papers presented the own fluorescence and phosphorescence of telomeres.

In this paper, the results of investigations of the optical absorption and the phosphorescence of a short telomere fragment – single-stranded oligonucleotide d(AGGGTTAGGGTTAGGGTTAGGG) (nucleotides in this oligomer are bound by phosphate groups PO_4H in $5'$ and $3'$ positions) under different temperature regiments of the preparation of samples and the detection of spectra are presented. These results give the ground to make conclusion about the nature of the traps for mobile triplet electronic excitations in telomeres. Moreover, the results obtained allow us to determine the space configuration of the traps of triplet excitations in the main part of the DNA macromolecule.

2. Experimental Procedure (Including Both the Preparation and Studying Methods)

Aqueous solutions of the investigated compounds (d(AGGGTTAGGGTTAGGGTTAGGG) oligonuc-

leotide and DNA macromolecule) with the concentration $C = 10^{-3}$ M are investigated. Optical absorption spectra were recorded using a Specord UV Vis spectrophotometer. The experimental accuracies were: for wave numbers (ν) – 50 cm^{-1}, for optical density (D) – 0.01. The optical absorption of two series of d(AGGGTTAGGGTTAGGGTTAGGG) samples was investigated: (1) intact sample; (2) the sample was preheated to 90 °C (with the aim to avoid the complexing between complementary chromophores of the different strands or the G-G-quadruplex forming – untwisted oligomer strand). Phosphorescence spectra were recorded at liquid helium temperature under the excitation in the range of wavelengths 240–300 nm, by using a spectrofluorimeter Hitachi MPF-4 with the time-resolving equipment. The experimental accuracies were: for wavelengths (λ) – 2 nm, for intensity (I) – 0.01. Phosphorescence of two series of d(AGGGTTAGGGTTAGGGTTAGGG) samples were investigated: (*1*) the sample was preheated to 90 °C (with the same aim as for optical absorption investigations – untwisted oligomer strand) and after a shock-freezing to the low temperature; (*2*) the sample was only shock-frozen.

3. Results and Discussion

3.1. Optical absorption

The optical absorption spectra of d(AGGGTTAGGGTTAGGGTTAGGG) oligomer (two types mentioned above) and DNA are presented in Fig. 1. It is shown that the absorbing centers in the investigated oligomer, as well as the DNA macromolecules, are the basic groups: the optical absorption spectrum curve of d(AGGGTTAGGGTTAGGGTTAGGG) is close to the additional sum of G-, T-, and A-basic groups. A little difference at $\lambda = 295$ nm is connected, possibly, with the absorption of G-G-quadruplex. With the aim to avoid G-G-quadruplex, the intact sample of d(AGGGTTAGGGTTAGGGTTAGGG) was heated to 90 °C and kept warm during the optical absorption spectrum recording. It is known that the oligomer strand at this temperature is untwisted, and G-G-quadruplex is damaged. It is shown that the band at $\lambda = 295$ nm practically disappears. The difference between the optical absorption spectra of d(AGGGTTAGGGTTAGGGTTAGGG) and DNA is connected with the absence of C-basic groups in the chemical structure of d(AGGGTTAGGGTTAGGGTTAGGG).

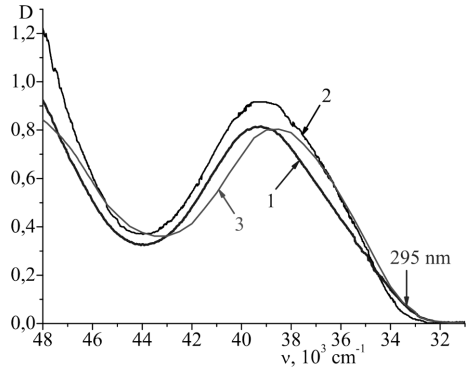

Fig. 1. Optical absorption spectra of: d(AGGGTTAGGGTTAGGGTTAGGG) (intact sample) (*1*), number 1 heated to 90 °C (untwisted) (*2*), DNA (*3*)

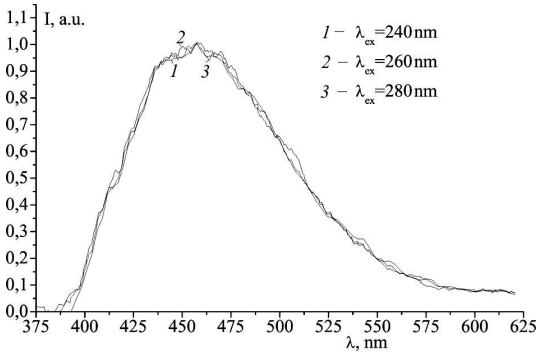

Fig. 2. Phosphorescence spectra at $T = 4.2$ K of d(AGGGTTAGGGTTAGGGTTAGGG) (intact sample) excited by: $\lambda = 240$ nm (*1*), $\lambda = 260$ nm (*2*), $\lambda = 280$ nm (*3*)

3.2. Phosphorescence

Two types of d(AGGGTTAGGGTTAGGGTTAGGG) oligomer samples (intact sample and untwisted) prepared by the method mentioned above are used to study the luminescence. The phosphorescence spectra at $T = 4.2$ K of the investigated compounds are presented in Figs. 2 and 3. The phosphorescence spectra of these two types of d(AGGGTTAGGGTTAGGGTTAGGG) in Fig. 4, as well as of d(CCCGGGTTTAAA) [1–3] in Fig. 3, are presented for comparison.

The phosphorescence spectra of d(AGGGTTAGGGTTAGGGTTAGGG) are close to the corresponding DNA spectra (within experimental errors) and practically do not depend on the excitation wavelength in the interval (240–

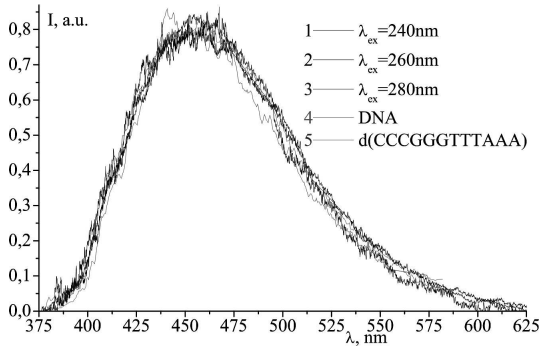

Fig. 3. Phosphorescence spectra at $T = 4.2$ K of d(AGGGTTAGGGTTAGGGTTAGGG) (preheated to 90 °C) excited by: $\lambda = 240$ nm (*1*), $\lambda = 260$ nm (*2*), $\lambda = 280$ nm (*3*); DNA (*4*) and d(CCCGGGTTTAAA) (excited by $\lambda = 260$ nm) (*5*)

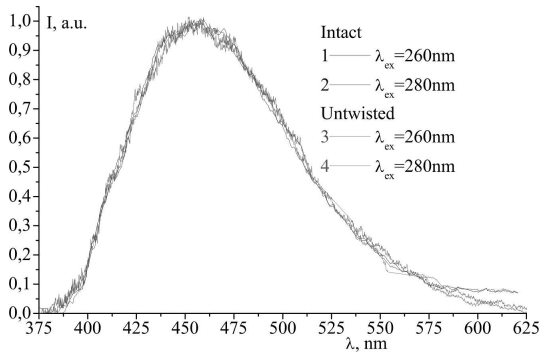

Fig. 4. Phosphorescence spectra at $T = 4.2$ K of d(AGGGTTAGGGTTAGGGTTAGGG) (intact sample (*1, 2*) and preheated to 90 °C (*3, 4*)) excited by: *1, 3* – $\lambda = 260$ nm, *2, 4* – $\lambda = 280$ nm

300 nm). This gives the ground to conclude that the main trap of triplet electronic excitations in d(AGGGTTAGGGTTAGGGTTAGGG), as well as in DNA and d(CCCGGGTTTAAA), is the complex formed by adenine (A) and thymine (T) chromophores. It is clear that, for the untwisted oligomer strand of d(AGGGTTAGGGTTAGGGTTAGGG), hydrogen bonds cannot be formed between the π-electron systems of the complementary nucleotides at the temperatures above 90 °C. Thus, one can conclude this AT-complex is formed by neighbor A- and T-chromophores from the same strand (not from A- and T-complementary chromophores of the different strands).

4. Summary

Thus, the main absorbing centers in d(AGGGTTAGGGTTAGGGTTAGGG) oligomer, as well as DNA macromolecules, are the individual nucleotides, because the optical absorption spectrum curve of d(AGGGTTAGGGTTAGGGTTAGGG) is close to the additional sum of G-, T-, and A-nucleotides. A little difference at $\lambda = 295$ nm is connected with the absorption of G-G-quadruplex according to [5, 6]. This proved by the disappearance of the band with maximum near $\lambda = 295$ nm under the heating of the telomere solution above 90 °C. The investigations of the phosphorescence show that the main traps of triplet electronic excitations in the telomere, as well as in DNA, are the complexes formed by neighbor adenine (A) and thymine (T) chromophores. Taking into account the fact of impossibility of the formation of hydrogen bonds between the π-electron systems of the nucleotides at temperatures above 90 °C, one can conclude this AT-complex is formed by neighbor A- and T-chromophores from the same strand (not from A- and T-complementary chromophores of the different strands). This conclusion is valid not only for telomeres, but for whole DNA macromolecules.

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СПЕКТРАЛЬНІ ВЛАСТИВОСТІ
ФРАГМЕНТІВ ТЕЛОМЕРИ

Р е з ю м е

У роботі наведені результати досліджень спектрів оптичного поглинання та фосфоресценції (при низьких температурах) фрагмента теломери – олігонуклеотида д(АГГГТТАГГГТТАГГГТТАГГГ) та (для порівняння) макромолекули ДНК при різних довжинах хвиль збуджуючого світла (240–300 нм). Досліджено два типи зразків д(АГГГТТАГГГТТАГГГТТАГГГ): (*1*) неушкоджений зразок та (*2*) зразок, нагрітий до 90 °С. Зроблено висновок про те, що пасткою триплетних електронних збуджень в олігонуклеотиді д(АГГГТТАГГГТТАГГГТТАГГГ), так само як і в ДНК, є комплекс, сформований між сусідніми аденіновою (А) та тиміновою (Т) хромофорами з одного і того самого ланцюга (але не між відповідними комплементарними хромофорами різних ланцюгів).