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Mobile electronic excitations in RNA

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Proofs have been obtained for the existence of mobile singlet and triplet electronic excitations in ribonucleic acid (RNA) macromolecule. They include: RNA phosphorescence and fluorescence quenching, phosphorescence and depolarized fluorescence of the excitation acceptor — cyanine dye Cyan40 bound with RNA while excited at the RNA's absorption maximum wavelength (λ_{ex} = 260 nm), and delayed fluorescence of the dye as a result of annihilation of the RNA mobile triplet excitations. The average singlet and triplet electronic excitation displacements along the RNA macromolecule were estimated.

Получены доказательства существования мобильных синглетных и триплетных возбуждений в макромолекуле рибонуклеиновой кислоты (РНК), такие как тушение фосфоресценции и флуоресценции РНК, фосфоресценция и деполяризованная флуоресценция акцептора возбуждения — связанного с РНК цианинового красителя Cyan40 при возбуждении на длине волны максимума поглощения донора — РНК (λ_{ex} = 260 нм), а также задержанная флуоресценция красителя, которая является результатом аннигиляции мигрирующих в РНК триплетных возбуждений. Произведена оценка средней длины пробега мобильных синглетных и триплетных электронных возбуждений вдоль макромолекулы РНК.

1. Introduction

Polynucleotide acids — DNA and RNA are the brightest examples of the natural functional materials. Their unique properties are defined mostly by their structure, interaction with the other cell components and intramolecular electronic-vibrational processes. The intramolecular electronic energy transfer is among the most important processes in DNA and RNA. Singlet electronic excitation transfer from a small donor molecule to the acceptor molecule incorporated artificially into RNA macromolecule is widely used in a number of biological and medical techniques for measurements of distances (usually 2 to 10 nm [1]) along the macromolecular chain [2-4]. In such structures RNA is considered as both an object under investigation and, at the same time, as an inert matrix embedding spatially separated donor/acceptor pairs. It is generally thought that electronic excitation transfer between two small molecules occurs via Forster mechanism, with RNA bases not involved since their energy levels are sufficiently higher [5]. Little is known, however, about excitation transfer (singlet as well as triplet) between RNA bases along the macromolecule. In a number of works it was shown that such migrating processes took place along the double helix DNA macromolecule, and the average displacement values were estimated [6-8]. In the present work, evidence of the existence of singlet and triplet excitation transfer in RNA macromolecule were obtained, and average displacements of these excitations were evaluated.

2. Experimental

Yeast RNA and cyanine dye Cyan40 were kindly provided by Institute of Molecular Biology and Genetics (IMBG) of NAS of Ukraine. Luminescence spectra were obtained at ambient and liquid nitrogen temperatures using a Cary Eclipse Spec-

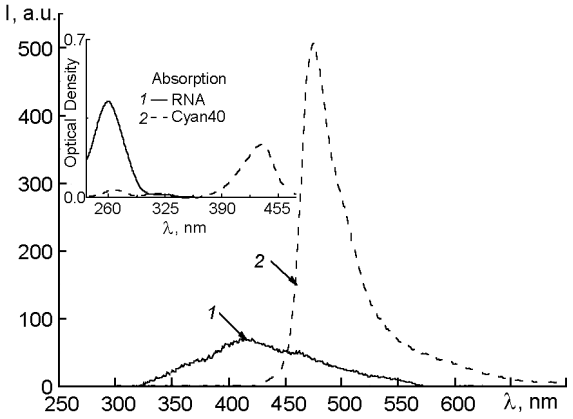

Fig. 1. Fluorescence spectra of *1* – RNA and *2* – RNA/Cyan40 (n = 5:1) at T=78 K, λ_{ex} = 260 nm. The insert: absorption spectra of *1* –RNA, $6 \cdot 10^{-5}$ M; *2* – Cyan40,$1.2 \cdot 10^{-5}$ M water solutions.

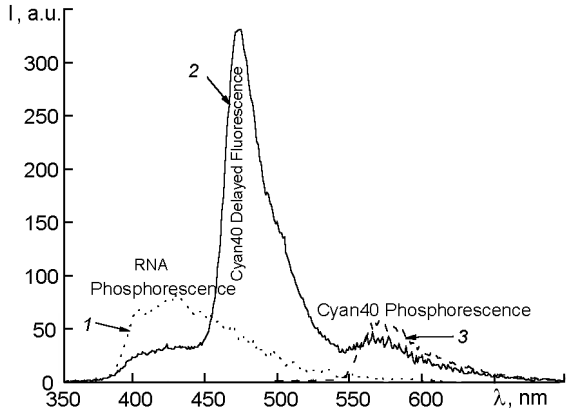

Fig. 2. Delayed emission spectra of RNA and RNA/Cyan40 mixture at T = 78 K, delay time relative to the excitation impulse 1 ms. *1* — RNA's phosphorescence, *2* — RNA/Cyan40 delayed emission (delayed fluorescence and phosphorescence), λ_{ex} = 260 nm, *3* — RNA/Cyan40 delayed emission, λ_{ex} = 435 nm.

trofluorometer (Varian Inc.) combined with an Optistat DN cryostat (Oxford Instruments). The absorption spectra were recorded using a Specord UV-VIS spectrophotometer.

3. Results and discussion

Dye Cyan40 was chosen as a trap for electronic excitation transfer along RNA. According to the authors of [9] this dye selectively bounds to RNA both *in vitro* and *in vivo*. This fact was also confirmed in our experiments: fluorescence quantum yield of the dye is increasing up to 140 times and the shift in its absorption spectra is appearing in the RNA/Cyan40 system as compared to the free dye.

3.1. Mobile singlet excitations in RNA

Some evidence of the intramacromolecular electronic excitation energy transfer was presented in our previous publication [5]. The main argument is domination of certain particular centers in the RNA luminescence spectra (all RNA bases absorb excitation light additively, but fluorescence spectra consists mainly of contributions from G (guanine) and C (cytosine) bases, while phosphorescence spectra reflect basically the emission of A (adenine) base possessing the lowest triplet electronic level [5, 10]).

In the current work, we studied migration of singlet excitations and their capturing by the non-covalently bound label Cyan40. The obtained results are: a) RNA fluorescence quenching upon adding of dyes-traps at different values of ratio n = 5:1, 10:1, 15:1, where n is the number of RNA bases per Cyan40 dye molecule (excitation at the RNA absorption maximum λ_{ex} = 260 nm, Fig. 1). When n = 30:1, the quenching effect significantly decreases, and intrinsic fluorescence of RNA reappears. It follows from these data that the displacement of singlet mobile excitations is less than the length of the 30 RNA bases sequence; b) depolarization of impurity fluorescence upon excitation in the RNA absorption band. The main reasons of this effect are rotation of the molecules or electronic excitation energy transfer [11]. In our case small molecules are fixed rigidly in RNA; thus, rotation of the probes is absent. Under excitation at the wavelength of 435 nm (absorption maximum of Cyan40), where RNA does not absorb (for the absorbtion spectra, see insert on Fig. 1), the values of fluorescence anisotropy are close to 0.2 for n = 5:1, 10:1, 15:1, 30:1. In the case of excitation in the absorption maximum of RNA the increasing of dye fluorescence anisotropy rate to 0.05 (n = 5:1, 10:1, 15:1) and 0.15 (n = 30:1) was observed, proving that the intramolecular singlet electronic energy transfer in RNA does exist. The fact of increasing of the fluorescence anisotropy for n = 30:1 gives the opportunity to estimate the average displacement of singlet mobile excitations along RNA in the range from 15 to 30 RNA bases.

3.2. Mobile triplet excitations in RNA

Besides data reported in [5], the intramolecular triplet energy transfer in RNA can be evidenced by: a) the RNA phosphorescence quenching under injection of Cyan40 molecules (Fig. 2); b) appearing of intensive delayed fluorescence of Cyan40 (forming a complex with RNA) under excitation at 260 nm and the absence of Cyan40 delayed fluorescence upon excitation at 435 nm. This suggested that not only transfer of triplet excitations takes place in RNA macromolecule, but also their annihilation when meeting each other. The phenomenon described above is observed even when the ratio $n = 30:1$. Therefore it can be concluded that the average displacement of mobile triplet excitation in RNA exceeds the length of 30 RNA bases (70 Å).

4. Conclusions

The intramolecular singlet and triplet electronic energy transfer in the RNA does really exist. The average displacement of the mobile singlet excitation is more than 15 and less than 30 RNA bases. The average displacement of the mobile triplet excitations was estimated to be more than 30 RNA bases.

For more accurate and detailed investigation of the mobile excitations in RNA, further studies are under way.

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Мігруючі електронні збудження в РНК

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Отримано докази існування мобільних синглетних і триплетних збуджень у макромолекулі рибонуклеїнової кислоти (РНК), такі як гасіння фосфоресценції та флюоресценції РНК, фосфоресценція та деполяризована флюоресценція акцептора збудження — зв'язаного з РНК ціанінового барвника Cyan40 при збудженні на довжині хвилі максимуму поглинання донора — РНК (λ_{ex} = 260 нм), а також затримана флюоресценція барвника, що є результатом анігіляції мігруючих в РНК триплетних збуджень. Оцінено середню довжину пробігу мобільних синглетних і триплетних електронних збуджень вздовж макромолекули РНК.