Electronic excitation energy transport in a DNA-Ag cluster complex.

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Abstract: We study electronic excitation transfer in the complexes of Ag clusters with oligonucleotides. Steady-state fluorescence excitation spectra show that the excitation energy is transferred to Ag cluster from all the 30 nucleobases of a 15-mer DNA duplex. This is in contrast to DNA-dyes complexes, where the energy transfer to the dye occurs from neighboring DNA bases only. Fluorescence decay curve for the DNA duplex shows that the process of energy transfer occurs within <100 fs. The obtained results suggest coherent excitonic type of the transfer rather than trivial Förster mechanism.

Introduction
Electronically excited states in DNA have been a subject of intense studies for a long time [1] due to their key role in the genetic damage induced by UV sun light. Such damage can lead to mutations, genomic instability, and carcinogenesis [2]. The underlying mechanism of carcinogenicity is associated with the direct absorption of a photon by DNA followed by cyclobutane pyrimidine dimer (CPD) formation [3]. In our recent theoretical study [4] we show that most DNA damages under terrestrial solar radiation occur on the stacked DNA bases with low-lying excitonic states. Such low-lying excited states might act as the traps in energy transfer process. Though energy transfer in DNA is clearly seen from fluorescence anisotropy decay even on femtosecond time scale [5], the range and mechanism of the transfer remain questionable. Dyes bound to DNA and modified nucleobases are often used as the acceptors in the experiments on energy and electron transport in DNA. Most experiments with intercalating dyes and chemically modified bases exhibited short-distance energy transfer only from neighboring DNA bases in the strand [6,7].

In present study, we used a new class of acceptors, namely metallic Ag nanoclusters, to study the process of energy transfer in DNA strand. Fluorescent DNA-bound Ag nanoclusters containing a few metallic atoms attract a lot of attention in last years because of their potential applications in nanotechnology and nanomedicine [8]. It has been shown that Ag clusters on short oligonucleotides were able to be rather efficient acceptors of the electronic excitation in the DNA-cluster complex [9]. This looks promising for developing a new type of ultraviolet to visible light converters, for example, for light emitting diodes [10].

We address the question about the range and mechanism of the excitation energy transfer in the complexes of DNA oligonucleotides with Ag clusters. We synthesized a green-emitting Ag cluster on a self-dimer of 15-mer of DNA 5’- CGCCCCCCTCGCGT-3’ and purified it by high performance liquid chromatography (HPLC) according to the previously described protocol [11]. From comparison of the absorption and fluorescence excitation spectra of the complex, we determined the number of DNA bases from which the energy was transferred to the cluster. The fluorescence life time of the donor, namely DNA duplex, was determined from the fluorescence up-conversion measurements on...
femtosecond time scale. Based on the observed high efficiency of the transfer on femtosecond time scale, we propose excitonic mechanism of the energy transport in the DNA-Ag cluster complex.

Results.
In Figure 1, shown are fluorescence emission, and excitation spectra of aqueous solution of freshly synthesized Ag clusters-DNA complex along with absorption spectrum of HPLC purified solution of the green-emitting Ag clusters. The fluorescence excitation spectrum of the complex coincides with the absorption spectrum within 10% experimental error. This fact clearly shows that the electronic excitation energy is transferred to the cluster from all 30 nucleobases of the duplex (as has been shown in ref. 11, the Ag cluster was stabilized by two DNA strands). The fluorescence decay curve of the 15-mer DNA duplex recorded at 330 nm in 5 ps time domain is shown in Figure 2. The excited state of major fraction of the DNA decays with a lifetime of ca. 300 fs. The energy was shown to be transferred from the whole DNA duplex with possible error of ca. 20%, i.e. with ≥80 % efficiency. At given rate of DNA deactivation of 300 fs it follows that the rate of the transfer should be faster than 100 fs.

Figure 1. Fluorescence emission and excitation spectra of Ag clusters bound to DNA duplex, and DNA absorption spectrum.
Although the detailed structure of the cluster-DNA complex is not known (we are currently working on it), some preliminary conclusions about possible mechanism of the energy transfer can be made. Based on high rigidity of the DNA duplex and small size of Ag cluster consisting of not more than 5 Ag atoms, as it follows from calculations of the excitation spectra of model Ag chains [12], it may be deduced that the distance of the energy transfer between DNA bases and Ag cluster comes to about 20-30 Å. Considering that the transfer occurs much faster than the DNA fluorescence life time of 300 fs, one may rule out trivial Förster mechanism. In this respect, the coherent (exciton) mechanism of the excitation transfer between DNA bases and Ag cluster seems the most probable. The result obtained in this study prompts further efforts in probing the ultrafast dynamics of the excitation in DNA and DNA-Ag cluster complexes in the time domain shorter than 100 fs, which is a challenging task for the deep UV range.

**Methods**

Synthesis and purification of Ag clusters were performed as describe in ref. 11. Ag clusters were synthesized by mixing DNA buffered solution (ammonium acetate buffer, pH 7) with AgNO3 and by reducing with NaBH4. The Ag/DNA base ratio was 0.4. Ion Pair-Reversed Phase HPLC of the green-emitting Ag cluster was performed on C18 column (Supelco Discovery 250 x 4.6 mm, 5 µm; gradient B in A 1-50% in 30 min; A – 70 mM triethylammonium acetate aqueous buffer (pH 7.5); B – 70 mM triethylammonium acetate solution in methanol).

Fluorescence spectra were obtained on Fluorolog 3 (Horiba Jobin-Yvon) fluorometer at room temperature. Fluorescent emission spectra were corrected for instrument sensitivity as supplied by manufacturers. To eliminate polarization effects, we set a vertically oriented polarizer into the excitation channel and another polarizer was placed at “magic” angle in the emission channel. The fluorescence excitation spectra were corrected for inner filter effect.
The fluorescence time-resolved measurements were made with FOG 100-DX fluorescence up-conversion spectrometer equipped with CDP 2015 third harmonic generator (CDP Corp., Moscow, Russia). The excitation was made by the third harmonic (266 nm) of a mode-locked Ti-sapphire laser (TISSA, CDP Corp., Moscow, Russia) operating at 80 MHz. The apparatus function (IRF) was determined by measuring the signal of Raman line in water. The FWHM of IRF was typically about 450 fs.

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