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Ultrafast energy transfer within pyropheophorbide-a tethered to self-assembling DNA quadruplex†

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Abstract

A DNA quadruplex system that exhibits ultrafast intramolecular energy transfer is discussed.

Purple non-sulfur photosynthetic bacteria use ring-arrayed bacteriochlorophylls as light-harvesting antenna to absorb photons and to initiate the photosynthetic process.¹ A number of chromophoric ring structures have been synthesized to probe the controlling features of energy transfer between pigments and to mimic the natural light-harvesting function in artificial photosynthetic constructs.^{2–7} The synthetic structures are composed typically of either covalently-linked^{4–10} or self-assembled cyclic chromophores.³ These systems have provided a means for achieving fast energy transfer in organic solvents. In the present work we report the preparation of a pyropheophorbide-a DNA conjugate and describe its ability to self-assemble into a circular arrangement through DNA quadruplex formation in aqueous buffer. Furthermore, we demonstrate that the quadruplex system exhibits ultrafast intramolecular energy transfer.

The oligonucleotide-tethered pyropheophorbides were prepared by coupling pyropheophorbide-a with ODNs tethered with a C6 amino modifier on a solid support on a 1 mM scale. The detailed synthesis of the modified oligo-nucleotides and the formation of DNA quadruplex are described in the ESI.[†]

The G-rich ODN (**1X**) tethered at the 5'-end with the pyropheophorbide-a (**X**) is shown in Fig. 1. **2X** is the pheophytin–DNA conjugate in which pyropheophorbide-a was tethered to a single strand incapable of forming a self-assembled structure. The secondary structures of the ODNs were confirmed by circular dichroism (CD) spectroscopy (Fig. 2). The functionalized quadruplex **1X** showed a positive ellipticity at 263 nm and a negative peak at 241 nm which is characteristic of tetramolecular parallel quadruplex formation.^{11–13} In contrast, **2X** only showed a weak peak at 273 nm and a negative peak at 249 nm in the CD spectrum, indicating no quadruplex formation.

[†]Electronic supplementary information (ESI) available: Experimental details and characterization of **1X** and **2X** along with transient absorption spectroscopy experiment and discussion. See DOI: 10.1039/b908435e

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The CD spectrum of **1X** confirms the formation of the DNA quadplex and the self-assembled non-covalent pyropheophorbide-a tetramer, but cannot reveal the relative orientations of the four pyropheophorbide-a rings nor reveal their ability to accomplish intermolecular energy transfer. To explore these issues, transient absorption spectroscopy coupled with modeling of the intermolecular energy transfer rates by hypothetical tetrameric ODN structures was performed.

The transient absorption spectra of **1X** and **2X** taken at different delay times after excitation into their lowest electronic excited state S_1 are shown in Fig. 3A and B, respectively. The spectra have typical chlorophyll-like shapes with very pronounced bleaching of the Qy band at ~680 nm, partially overlapped with stimulated emission bands at ~685 and 740 nm, and a broad, positive $S_1 \rightarrow S_n$ transient absorption band in the 560–660 nm range. **1X** shows a very fast partial recovery component of the bleaching of the Qy band (Fig. 3A). This component is not observed for **2X** where instead, the spectral bleaching persists for tens of picoseconds (Fig. 3B).

In order to determine whether the fast component of the recovery of the ground state bleaching in the aggregated chromophore system **1X** was due to singlet–singlet annihilation, the dependence of the dynamics on laser intensity was examined at 688 nm (Fig. S3, ESI[†]). The figure shows clearly that the recovery of the ground state bleaching is dependent on laser intensity. Therefore, the time constant for energy transfer between adjacent chromophores, τ_h , can be approximated from the equation $\tau_h = \tau_a 8 \sin^2(\pi/2N)$ which expresses the energy transfer time as a function of the singlet annihilation lifetime, τ_a , for cyclic aggregates with N molecules having identical microscopic energy transfer rates.^{3,14}

The fluorescence lifetime of monomeric pyropheophorbide-a chromophore (**2X**) was obtained using single photon counting fluorescence spectroscopy (TCSPC) (Fig. S4, ESI[†]), and measured to be 5.1 ns. This value was subsequently used as a fixed parameter in the global fitting analysis for the transient absorption datasets taken from **1X** and **2X** (Fig. S5 and S6, ESI[†]). Global fitting of the spectral and temporal datasets of **1X** revealed two fast kinetic components having lifetimes of 540 fs and 5.5 ps (Fig. 4). Global fitting of the dataset from **2X** shows only one fast kinetic component with a lifetime of 5.2 ps which is very similar to the value of 5.5 ps observed for **1X**. This component is not sensitive to laser intensity and is probably associated with the spectral equilibration of vibrational energy following excitation. The intensity dependent kinetic component seen in **1X** has a lifetime of 540 fs which corresponds to a τ_h value of 630 fs or a rate constant, $k_h = 1/\tau_h = 1.58 \times 10^{12} \text{ s}^{-1}$.

The modeling of the energy transfer rate constant within the tetrameric system was performed on the basis of idealized tetrameric structures. The size of the guanosine DNA tetraplex was assumed to be 11 Å, based on the diameter of pyropheophorbide-a ring (Fig. S7, ESI[†]). It was also assumed that the tetrameric ring array of **1X** adopts a cubic conformation (Fig. S8, ESI[†]) with a slightly larger side dimension of 14 Å to account for the distance of closest approach of the rings on the faces of the cube.

Three models having different chromophore transition dipole moment orientations (shown in Fig. S8, ESI[†]) were analyzed. Model **1XA** (Fig. S8, ESI[†]) predicts identical nearest neighbor microscopic rates of $11.8 \times 10^{12} \text{ s}^{-1}$ and no crossdiagonal energy transfer (Table S1, ESI[†]). Model **1XB** (Fig. S8, ESI[†]) predicts two different nearest neighbor microscopic rates ($11.8 \times 10^{12} \text{ s}^{-1}$ and $47 \times 10^{12} \text{ s}^{-1}$) and also a cross-diagonal microscopic energy transfer rate of $94 \times 10^{12} \text{ s}^{-1}$ (Table S1, ESI[†]). Model **1XC** (Fig. S8, ESI[†]) predicts the same three microscopic rates as **1XB**, but in a different order (Table S1, ESI[†]). Model **1XA** best agrees with the experimental observation of a mono-exponential energy transfer time. In this

model, the Förster energy transfer rate constant of $11.8 \times 10^{12} \text{ s}^{-1}$ corresponds to an 85 fs transfer time which is over six times faster than the experimentally observed time of 530 fs. Nevertheless, model **1XA** is the only one that would not be expected to show multi-exponential kinetic behavior, and therefore, it is likely to be a reasonable approximation to the structure of the assembled tetramer. Variations from the model maybe due in part to differences in the inter-chlorophore distance from the idealized cubic structure used in the calculation. Also, our modeling is simple, and various processes occur simultaneously in the time-resolved experiments, which makes it very difficult for comparison of experiments.

In summary, we have shown that **1X** forms a self-assembled pyropheophorbide-a tetramer–DNA quadruplex system that displays ultrafast intramolecular energy transfer in aqueous buffer. Future work will explore the controlling features of energy transfer by altering the pheophytin structures or the length of the linker used to tether the DNA quadruplex to the pheophytins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

(a) Hypothetical parallel tetramolecular G-quadruplex with pyropheophorbide fragment. (b) Sequences of the designed oligonucleotides wherein G-quadruplex **1X** is tethered with pyropheophorbide fragments, and **2X** is a single strand-pyropheophorbide adduct that is incapable of forming quadruplex.

Fig. 2. Circular dichroism spectra of ODNs after being subjected to the self-assembly conditions. All measurements were done in 80 mM KCl, 10 mM Tris-HCl, pH 7.5 buffer. Concentrations of all ODNs were 40 μ M for the single strand.

Fig. 3.
(A) Transient absorption spectra of pyropheophorbide-a tethered DNA quadruplex (**1X**) and
(B) transient absorption spectra of DNA single strand-pyropheophorbide-a adducts (**2X**)
obtained after 1 μ J excitation at 677 nm. Representative spectra were taken at different times
following excitation. All measurements were done in 80 mM KCl, 10 mM Tris-HCl, pH 7.5
buffer at room temperature.

Fig. 4. Transient absorption kinetic traces taken at 688 nm of pyropheophorbide-a tethered DNA quadruplex (**1X**, in black) and single strand-pyropheophorbide DNA (**2X**, in red) obtained after 1 μ J excitation at 677 nm. Solid bold lines represent fits obtained from global fitting analysis (see ESI[†]). The amplitudes of the traces were normalized to each other at time zero.