Efficient Upconversion by Highly Water-Soluble Cationic Sensitizer and Emitter in Aqueous Solutions with DNA

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ABSTRACT: Highly water-soluble cationic palladium porphyrin as a sensitizer and 9,10-bis(4-trimethylammoniumphenyl)anthracene as an emitter were newly synthesized. They were shown to be bound and immobilized in DNA double helix assembly from absorption, fluorescence, phosphorescence, and circular dichroism spectra. Upon excitation at 532 nm in deaerated aqueous solutions, they showed weak blue upconversion fluorescence, the efficiency of which increased dramatically in the presence of DNA. The threshold power density between the second-order and first-order power dependence of upconversion fluorescence decreased to less than a half upon addition of DNA. The emitter triplet lifetime estimated from time dependences of upconversion fluorescence at low power ns pulsed laser was found to considerably increase in the presence of DNA. From these results, DNA was concluded to work effectively in concentrating both sensitizer and emitter and in migrating excited triplet states, resulting in efficient upconversion.

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INTRODUCTION

Upconversion (UC) based on triplet energy transfer and triplet−triplet annihilation (TTA) by the use of two kinds of molecules, one (the sensitizer) absorbing at longer wavelengths and one (the emitter) with emission at shorter wavelengths, has attracted much attention for its potential applications in solar cells and bioimaging. Sensitized delayed fluorescence from anthracene was observed more than 50 years ago in solutions of phenanthrene containing traces of anthracene.1−5 But it was not regarded as TTA-UC until Balaschev et al.4 reported upconversion photo-emission in polyfluorene film doped with platinum octaethylporphyrin in 2003. Since then more than 350 papers have been published in TTA-UC. In almost all cases, hydrophobic or lipophilic sensitizers and emitters have been used which are soluble only in organic solvents such as toluene, N,N-dimethylformamide, dichloromethane, acetonitrile. For bioimaging applications and from an ecological point of view, they are not desirable. Efforts have been made to use these lipophilic sensitizers and emitters in aqueous media by employing micelles or polymer micelles,5−6 microcapsules,8−10 microemulsions,11,12 vesicles or liposomes13−19 to solubilize them in hydrophobic region of microdomains.

Only one report has been made so far for TTA-UC by a watersoluble anionic sensitizer and an amphiphilic cationic emitter.20 Amphiphilic diphenylanthracene derivative with two decyltri-
methylammonium groups through amide bonds at para-position of phenyl group formed monolayer self-assemblies in water.20 UC was not observed by adding anionic platinum tetracarboxyphenylporphyrin as a sensitizer to such monolayer self-assemblies in water. To observe clear UC for a mixed aqueous dispersion of anionic platinum tetracarboxyphenylporphyrin with cationic monolayer self-assemblies, microwave heating at 393 K for 30 min and incubation at room temperature with stirring for 48 h, was found necessary.20 DNA is water-soluble and is well-known to immobilize various molecules by intercalation between planar base pairs of the double helix, by groove binding and by electrostatic binding, DNA can thus be used as a host with well-ordered and rigid structure to concentrate dyes and control their physical properties. We have utilized DNA ultrathin films for very sensitive detection of nitrogen oxide gases through electron transfer quenching of fluorescent dyes immobilized in the double helix.21 We have also demonstrated highly efficient energy transfer and excitation energy migration along the DNA double helix.22,23

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UC fluorescence in DNA-based assembly was recently reported by tris(2,2'-bipyridine)ruthenium, [Ru(bpy)_3]^{2+}, as a sensitizer and (R)-1-O-[4-(1-pyrenyl- ethynyl)phenylmethyl]-glycerol, PEPy, chemically attached at the 3' and 5' positions of oligonucleotides with 12 base pairs as an emitter, either one or two units at each position. It was reported that UC fluorescence was observed for three kinds of emitter-modified oligonucleotides and [Ru(bpy)_3]^{2+} in aqueous solutions aerated with argon for 24 h, whereas no UC was observed in dichloromethane solutions of [Ru(bpy)_3]^{2+} and PEPy in the same conditions. Similar DNA-based assembly with zinc meso-tetrakis(N-methylpyridinium-4-yl)porphyrin as a sensitizer and PEPy as an emitter did not result in UC fluorescence.

In the present study, we employed a highly water-soluble cationic sensitizer and emitter and achieved efficient TTA-UC in aqueous solutions from them by just adding DNA.

**EXPERIMENTAL METHODS**

**Materials.** Water-soluble sensitizer, palladium meso-tetrakis(N-methylpyridinium-4-yl)porphyrin, PdTMPyP, was prepared by refluxing aqueous solutions of free base form with sodium tetrachloropalladate. 9,10-Dibromoanthracene was coupled with 4-(dimethylamino)-phenylboronic acid in the presence of tetrakis(triphenylphosphine)palladium and cesium carbonate to form 9,10-bis[4-(dimethylamino)-phenyl]anthracene. 9,10-Bis[trimethylammonium-4-yl]-phenyl]-anthracene (abbreviated as TAPA) was obtained by reacting it with methyl iodide. Counter ions of both final products were exchanged to chloride by passing through an ion exchange column. Details of synthesis, purification and characterization are given in the Supporting Information. Deoxynucleobase acid (DNA) sodium salt from salmon testes (D1626, Sigma-Aldrich) was used as received.

Aqueous solutions of 0.01 mM PdTMPyP with 0−0.5 mM TAPA were prepared in the absence and presence of 0.4 mM DNA based on an average molecular weight of base pairs using deionized water.

**Measurements.** Absorption spectra, fluorescence and phosphorescence spectra were measured with JASCO V-770 spectrophotometer and Hamamatsu Photonics C10083CAH high resolution mini-spectrometer, respectively. UC measurements were made for deaerated samples by repeated freeze-pump cycles upon excitation with MGL-III-532-10 mW green TPPS laser (CNI Optoelectronics Technology, China) through NDHN-50 variable ND filter (SIGMA KOKI, Japan). The emission was observed through NF-2SC05-40-532 notch filter (SIGMA KOKI, Japan) and an optical fiber by Hamamatsu Photonics C10083CAH high resolution mini-spectrometer or PMA-11 Photonic Multichannel Spectral Analyzer. The UC quantum yield was evaluated by the relative method using cresyl violet as a standard in a similar manner as for UC measurements. Time-resolved UC measurements were made by a system composed of a Q-switched Nd:YAG laser, a monochromator equipped with a photomultiplier and a digital oscilloscope. Circular dichroism (CD) spectra of PdTMPyP and TAPA in aqueous solutions with DNA were measured by JASCO J-820 CD spectrometer at room temperature.

**RESULTS AND DISCUSSION**

Structures of PdTMPyP 1 and TAPA 2 are shown in Figure 1 together with their absorption spectra in aqueous solutions without (blue line) or with (red line) DNA (0.4 mM). Both spectra showed red-shift and hypochromic effects in the presence of DNA. Emission spectra of PdTMPyP 1 (0.005 mM) in deaerated aqueous solutions with 0.2 mM DNA (red line) and without DNA (blue line) are shown in Figure 2. In the presence of DNA, the phosphorescence peak is red-shifted from 690 to 706 nm and its intensity increases approximately 5-fold. The corresponding fluorescence peak shows a similar red-shift but little change in intensity. These results together with CD spectra shown in Figures S3 and S4 clearly indicate that both cationic dyes are immobilized in double helix DNA assembly either by intercalation, groove-binding or electrostatic binding in accordance with previous reports.

Phosphorescence from 1 at 690 nm was quenched by 2 in aqueous solutions without DNA as shown in Figure S5 with a Stern−Volmer constant, $k_{SV} = 5.1 \times 10^3 \text{M}^{-1}$. In the presence of DNA, phosphorescence from 1 at 706 nm was quenched more effectively by 2 as shown in Figure S6, which gave $k_{SV} = 1.6 \times 10^4$...
M⁻¹. These results strongly suggested that triplet energy transfer from 1 to 2 was facilitated by concentration and/or immobilization of 1 and 2 in DNA double helix assembly. These results indicate that energy transfer from triplet state of cationic 1 to the cationic emitter 2 occurred in aqueous solutions with or without DNA and that its efficiency was increased in the presence of DNA. Upconversion fluorescence from 2 was observed in concomitant quenching of phosphorescence of 1 as shown in Figures S5 and S6, the intensity of which depended on the concentration of 2 and/or the presence of DNA.

Similar effects of DNA were more remarkably observed in UC fluorescence as shown below. Emission spectra for 1 (0.01 mM) and 2 (0.2 mM) in aqueous solutions are shown in Figure 3 (a) with DNA (0.4 mM) and (b) without it at various excitation powers. UC fluorescence with a peak at 450 nm in the presence of DNA is fairly strong at this concentration (0.01 mM sensitizer; 0.2 mM emitter) with 820 mW cm⁻² excitation power, and is about 80.4% of corresponding phosphorescence at 705 nm. Meanwhile, UC fluorescence with a peak at 435 nm is extremely weak in aqueous solutions without DNA, only about 4.1% of phosphorescence at 691 nm at the same condition. By increasing the ratio of the sensitizer and emitter to 1:50, UC fluorescence can be clearly observed even without DNA, though its intensity is much lower than that observed in the presence of DNA. The power density dependences of phosphorescence at 705 nm and UC fluorescence at 450 nm for 1 (0.01 mM) and 2 (0.5 mM) in aqueous solutions with DNA (0.4 mM) are shown in Figure 4a. The slope (0.84) of the double-logarithmic plot of phosphorescence emission intensity vs power density is close to the expected value of unity. For UC fluorescence the corresponding plot has a slope of 1.18 for power densities higher than about 150 mW cm⁻², but is 1.65 for lower power densities. At the same concentrations of 1 and 2 in aqueous solutions without DNA, the observed UC fluorescence intensity at 435 nm was much weaker as shown in Figure 4b. It was only about 10–20% of that with DNA, depending on the power density. In contrast with the slope of 0.94 for phosphorescence in all power ranges, that for UC fluorescence is 1.21 in the higher power region above about 300 mW cm⁻², but 2.22 below that power.

It is known that UC fluorescence intensity has a quadratic dependence on excitation power as expected for bimolecular reactions when the power is low.²⁹⁻⁻³² This is the case when k₂T ≫ kTT[E*]₀, where k₂T is the first-order rate constant of emitter excited triplet 3E*, kTT is the second-order rate constant corresponding to TTA, and [E*]₀ is the initial concentration of E*. The UC fluorescence intensity dependence on excitation power approaches linear when k₂T ≪ kTT[E*]₀, because [E*]₀ becomes high enough.³³ Our observed results indicate that the threshold distinguishing the deactivation rate of emitter excited triplet state, either the first-order kinetics or the second-order triplet—triplet annihilation reaction, can be controlled by DNA. As shown in Figure 4a, b, DNA clearly lowered the threshold in aqueous solutions, which strongly suggested that the lifetime of emitter excited triplet state becomes longer or k₂ becomes lower in the presence of DNA.

The quantum yield, ΦUC, was measured to describe the positive effect of DNA on TTA-UC quantitatively. The ΦUC-value for aqueous solutions without DNA is very low, 0.0013%, due most probably to Coulomb repulsion between positive charges of both sensitizer and emitter. It should be noted that the ΦUC-value increases approximately 30-fold in the presence of DNA as shown in S.I. This enhancement by DNA well corresponds with the increase of emitter triplet lifetime as mentioned below. The detailed estimation procedure and results are given in S.I. These results clearly indicated the positive effects of DNA on TTA-UC, although more efforts are needed to further increase the absolute quantum yield.
The dynamics of phosphorescence from 1 and UC fluorescence from 2 were studied in aqueous solutions with and without DNA upon excitation at 532 nm with nanosecond (ns) pulsed laser. The phosphorescence lifetime of 1 in aqueous solutions with DNA is significantly longer than that without DNA. The UC fluorescence lifetime is also prolonged in the presence of DNA. These findings suggest potential applications in sensing and imaging.
solutions was increased to 60 μs (31.4%) and 172 μs (68.6%) in the absence of DNA in comparison to 32 μs (100%) in the absence of DNA as shown in Figure 5a, b. The phosphorescence longer lifetime observed in the presence of DNA well corresponded to the increase of phosphorescence intensity as mentioned above (Figure 2) upon addition of DNA. Figure 5b clearly indicated the first-order decay of excited triplet of 1 in aqueous solutions without DNA. Meanwhile two components with longer lifetimes were observed in the presence of DNA as shown in Figure 5a, which are most probably due to different immobilization states of 1 in DNA such as electrostatic binding, groove binding, or intercalation. The Stern–Volmer constant \( k_{SV} \) mentioned above equals to \( k_{q} \tau_{q} \), where \( k_{q} \) is the second-order quenching constant of phosphorescence by 2 and \( \tau_{q} \) is the phosphorescence lifetime of 1. The \( k_{q} \) values were estimated to be \( 1.6 \times 10^{8} \) and \( 1.2 \times 10^{8} \) M\(^{-1}\)s\(^{-1}\) based on \( \tau_{q} = 32 \) μs in the absence of DNA and 137 μs (average value) in the presence of DNA, respectively. The estimated values are of the same order of magnitude but slightly smaller than those reported for neutral sensitizer and emitters in organic solutions.12 These results indicated that cationic characters of both 1 and 2 together with immobilization in DNA double helix assembly, as shown by longer lifetime and higher phosphorescence intensity, have little disturbing effect on triplet energy transfer.

Time dependences of UC fluorescence for 1 (0.01 mM) and 2 (0.5 mM) are shown in Figure 6 for aqueous solutions (a) with DNA (0.4 mM) and (b) without it. In the presence of DNA, strong UC fluorescence was observed at lower power, 0.4 mJ/pulse, to give a single exponential decay with a lifetime (τ) of 34.6 μs. A little higher power, 1.0 mJ/pulse, was needed to observe the dynamics in the absence of DNA due to very weak signals, which gave a shorter component with \( \tau = 1.35 \) μs (86.6%) and a longer one, \( \tau = 6.4 \) μs (13.4%). The triplet lifetime of emitters, \( k_{T}^{-1} \), is rather than to twice of the UC fluorescence lifetime \( \tau \) observed with excitation power as low as possible. According to the analytical solution of the rate equation composed of the first- and the second-order kinetics for excited triplet emitters,31,33 the time dependence of emitter triplet concentration is expressed by eq 1 when \( k_{T} \gg k_{q}[^{3}E^{*}]_{0} \) at low excitation powers, where \( k_{T} \) is the first-order rate constant of emitter excited triplet \( ^{3}E^{*} \), \( k_{q} \) is the second-order rate constant corresponding to TTA, and \( [^{3}E^{*}]_{0} \) is the initial concentration of \( ^{3}E^{*} \).

\[
[^{3}A^{*}] = [^{3}A^{*}]_{0} \exp(-k_{T}t)
\]

Then, time-dependent TTA-UC fluorescence is expressed by eq 2,

\[
I_{UC}(t) \propto \frac{\Phi_{[^{3}E^{*}]}}{2k_{T}}[^{3}A^{*}]_{0} \exp(-k_{T}t)^{2}
= \frac{\Phi_{[^{3}E^{*}]}}{2k_{T}}[^{3}A^{*}]_{0}^{2} \exp(-2k_{T}t)
\]

The lifetime of UC fluorescence is defined from this equation by

\[
\tau_{UC} = \frac{1}{2k_{T}}
\]

Therefore, the emitter triplet lifetime, \( \tau_{T} \), is given by eq 4.

\[
\tau_{T} = \frac{1}{k_{T}} = 2\tau_{UC}
\]

The triplet lifetime of 2 was thus evaluated to be 69.2 μs (100%) in the presence of DNA, 2.7 μs (86.6%), and 12.8 μs (13.4%) in the absence of DNA. DNA was thus found to increase the triplet lifetime of emitter 2 by 5.4–25.7 times, which most probably contributed to highly efficient UC fluorescence and lower threshold power in aqueous solutions with DNA by cationic sensitizer and emitter observed in the present study for the first time. Because the concentration of 2 is 1.25 times that of average base pairs of DNA, all base pairs will contain an emitter 2 and one out of four base pairs contain two emitter molecules. On the basis of the distance between base pairs, 0.34 nm,34 the average distance among emitters on DNA backbone is estimated to be equal to it or less. Energy migration among emitters well-organized and immobilized in DNA double helix assembly23,25 is presumed to contribute to such positive effects.

Schematic representation of efficient TTA-UC from highly water-soluble cationic sensitizer and emitters, which are concentrated and immobilized in DNA double helix assembly, is shown in Figure 7.

**CONCLUSIONS**

Considerable enhancement of UC fluorescence was observed using highly water-soluble cationic sensitizer and emitter, for the first time, in aqueous solutions with DNA. From spectroscopic data, excitation power dependence and dynamic results, it was concluded that DNA concentrated and immobilized these dyes into double helix assembly and facilitated triplet energy transfer and triplet–triplet annihilation.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsbiomaterials.7b00238.

Figures S1–S6 (PDF)

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Notes

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