Acceleration of Guanine Oxidation under Visible Light Irradiation by Photon Upconversion Based on Triplet–Triplet Annihilation

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ABSTRACT

We report the fluorescent polymer complex which can show fluorescence emission at 380 nm with the excitation of 520 nm in aqueous media. This photon upconversion based on triplet–triplet annihilation can efficiently take place via inter-molecular energy transfers between the Ru complex as a sensitizer and anthracene molecules as an emitter captured into the water-soluble network polymers. We performed the oxidation reaction of 2'-deoxyguanosine by riboflavin in the presence of the polymer complex with the visible light irradiation. It was clearly indicated that oxidative decomposition can be accelerated by UV light generation via upconversion based on triplet–triplet annihilation.

INTRODUCTION

Photoreactions are powerful methods to visualize and regulate biological events in the cells or vital body. Various kinds of molecular probes such as environmental-responsive fluorophores, photo-therapeutic anticancer drugs, and photo-manipulation systems of biomolecules have been developed, and some of them have been clinically used. In order to proceed photoreactions in high yields, short-wavelength light is favorable. However the photo-degradation of the drugs or the probe molecules and the damages to living organisms will take place under biological conditions. In addition, decay of light through vital organs should be considerable issue in the use of short-wavelength light for investigating deep positions inside body.

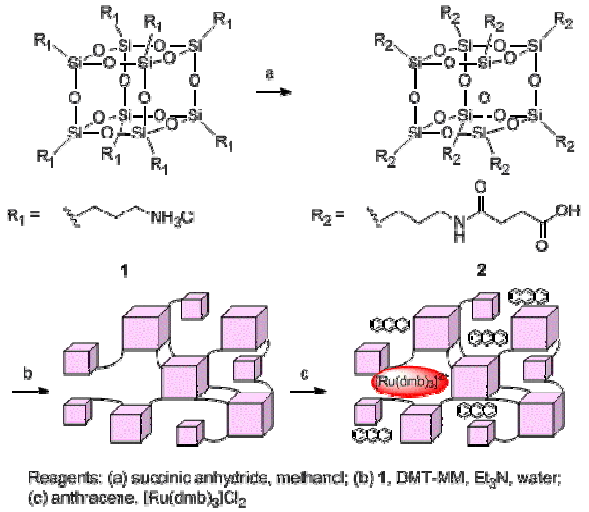
Upconversion (UC) to generate shorter-wavelength light emission than that of excitation is one of solves to overcome these problems. Indeed, the multi-photon step has been already applied for biotechnical analyzer, such as multi-photon microscopy. However, large power and coherent light should be required to raise multi-photon excitation. In contrast, UC via triplet–triplet annihilation (TTA) can proceed with weak power and non-coherent light such as sun light.[1] Several groups have reported UC using nanoparticles for bioimaging.[2] On the other hand, very few were the applications of TTA-supported UC process to the photoreaction with biomolecules.

Herein, we present TTA-supported UC system which can show fluorescence emission at 380 nm with the excitation of 520 nm in the aqueous phase using the polymer complex. TTA-supported UC can efficiently occur in water via inter-molecular energy transfers between the Ru complex as a sensitizer and anthracene molecules as an emitter captured into the water-soluble network polymers. The oxidation reaction of 2'-deoxyguanosine (dG) by riboflavin was performed under visible light irradiation in the presence of the polymer complex, and it was confirmed that oxidative decomposition can be accelerated via UC. This is the first example, to our knowledge, for developing the water-soluble polymer material which can show anti-Stokes fluorescence and applying TTA-supported UC to the photochemical reaction with biomolecules in aqueous media.

RESULTS AND DISCUSSION

We used the metal-to-ligand charge transfer sensitizer $[Ru(dmb)_3]^{2+}$ (dmb = 4,4'-dimethyl-2,2'-bipyridine) and anthracene, for which the green-to-blue UC can be readily visualized by the naked eye in a lighted room without using a laser.[1] On the other hand, anthracene shows extremely poor water-solubility, and for accomplishing an effective photon UC process, $[Ru(dmb)_3]^{2+}$ and multiple anthracene molecules should be gathered closely. In order to solve these problems, we designed the water-soluble polymer complex, which encapsulated $[Ru(dmb)_3]^{2+}$ and anthracene. We adopted the network polymer consisting of cubic silsesquioxane, POSS, as a scaffold of the complex.[3] The POSS polymer shows good water-solubility and can work as a host to encapsulate hydrophobic molecules.[4] Therefore, the POSS-based polymer complex can be expected to capture and maintain the assembly of $[Ru(dmb)_3]^{2+}$ and anthracene in the aqueous solutions.

Scheme 1 illustrates the synthesis of the POSS polymer complexes. Octa-substituted amino- and carboxy-termini POSS were condensed in water, and the water-soluble white powders were obtained after purification.[3] The samples containing 1 mg/mL of the POSS polymers, 100 μM anthracene and 1 μM $[Ru(dmb)_3]^{2+}$ in 50 mM sodium phosphate buffer (pH = 7.0) were sonicated for 15 min at 25 °C for entrapping to the polymers. The polymer complex obtained here was used for following measurements.



Reagents: (a) succinic anhydride, methanol; (b) **1**, DMT-MM, Et_3N, water; (c) anthracene, $[Ru(dmb)_3]Cl_2$

Scheme 1. Synthetic scheme of the POSS polymers and the complex formation.

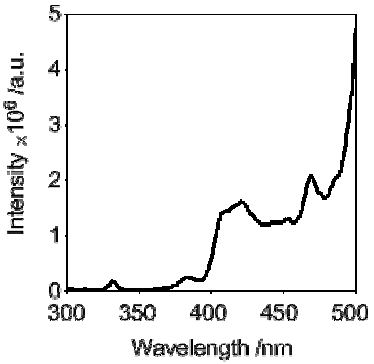


Figure 1. Fluorescence spectrum of the solution containing 1 mg/mL of the POSS polymer, 100 μM anthracene, 1 mM $[Ru(dmp)_3]Cl_2$ in 50 mM sodium phosphate buffer (pH = 7.0) with the excitation at 520 nm.

Figure 1 represents the fluorescence spectrum of the sample solution containing above polymer complex in 50 mM sodium phosphate buffer (pH = 7.0) with the excitation at 520 nm. Significant emission between 370 nm to 450 nm assigned as the emission bands of the singlet state of anthracene was observed from the aqueous solution of the polymer complex. These data clearly indicate that TTA-supported UC was accomplished in the aqueous solution.

Oxidation of guanine has great importance not only as one of major processes of DNA damages and carcinogenesis but also as an initial step in the hole transport reaction through DNA. Riboflavin can work as a photosensitizer to proceed one-electron oxidation of guanine under UV (365 nm) irradiation, and subsequent degradation mechanism was investigated.[5] We evaluated photo-triggered guanine oxidation by riboflavin in the presence of the polymer complex under the light irradiation (Figure 2). Reaction yields were evaluated from the peak area in HPLC profiles. Under the visible light irradiation

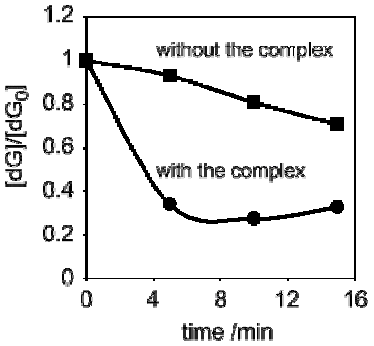


Figure 2. Time-course of the concentration of dG during the photoreaction by riboflavin (100 μM) with a transilluminator (480 nm ± 20 nm) in the presence (circular dots) or absence (square dots) of the polymer complex in 50 mM sodium phosphate buffer (pH = 7.0) at 0 °C.

(480 nm), the amount of dG in the sample was slightly changed, compared to the sample without irradiation. Significantly, dG was converted to dIz within 15 min only in the sample containing riboflavin and the polymer complex under the visible light irradiation. These results indicate that visible light irradiation to the polymer complex accelerated guanine degradation in the presence of riboflavin via UV generation by TTA-supported UC from the polymer complex.

CONCLUSION

In conclusion, the water-soluble polymer complex which can convert visible light to UV light was constructed, and oxidative decomposition of dG in the presence of riboflavin, which conventionally proceeded under UV light irradiation, can be accelerated under visible light irradiation via UC based on TTA using this polymer complex. Our system might be applicable for photochemical reactions under biological conditions. We have continuously challenged to apply photon UC systems to the photochemical reactions in vivo.

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