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Nanoparticle-mediated singlet oxygen generation from photosensitizers

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ABSTRACT

We report on the modification of the efficiency of singlet oxygen generation in photosensitizers conjugated to dielectric (CeF_3) and metal (Au) nanoparticles in water. The conjugates were formed with two photosensitizers, verteporfin and Rose Bengal. Quantitative analysis of the singlet oxygen generation demonstrated that the conjugation of the photosensitizer to a nanoparticle increases the efficiency of the photosensitizers to produce singlet oxygen in water. The singlet oxygen quantum yield of UV-sensitized verteporfin increases by a factor of 1.45 and 1.64 for CeF_3-verteporfin and Au-verteporfin conjugates respectively compared to unconjugated verteporfin. Furthermore, Au-Rose Bengal conjugates also demonstrated enhanced singlet oxygen yield when sensitized at the plasmonic wavelength of 532 nm. We quantitatively explain these findings by the electric field enhancements around the nanoparticles.

1. Introduction

Photodynamic therapy (PDT) is a clinically approved treatment modality extensively used in both oncological and other therapies [1,2]. PDT is based on the concept that the photosensitizer (PS) molecules accumulate in the tissues upon systemic administration. They are then sensitized by light at an appropriate wavelength directly at the treated site. This triggers a cascade of events starting from absorption of light by a photosensitizer which then transfers its charge or energy from its triplet state to adjacent biomolecules (type I) or to molecular oxygen (type II) to create an activated singlet oxygen molecule ($^1O_2$) [3–5] and/or other highly reactive oxygen species (ROS). These ROS are a cytotoxic and able to kill cancer as well as microbial cells [6] when sufficiently abundant. In order to detect the singlet oxygen generation, different approaches including direct methods and chemical probe methods have been used [7,8]. Singlet oxygen Sensor Green (SOSG), the probe used in this work is a highly specific probe specifically designed for detecting the 1O2 compared with other ROS [9].

The efficiency of the photosensitizer to generate singlet oxygen is measured by its singlet oxygen quantum yield (SOQY). SOQY is the ratio of number of photons absorbed by the PS to the number of generated singlet oxygen molecules. Among different methods to determine the SOQY of photosensitizers the reference method is the most common [10]. In this approach, the SOQY of the PS under optical irradiation, ϕ_{PS}, can be determined by calibration against a reference photosensitizer (REF) with a known SOQY ϕ_{REF}:(See Supplementary information S1)

$$\phi_{PS} = \phi_{REF}\frac{\frac{r_{PS}}{(1-T_{PS})}}{\frac{r_{REF}}{(1-T_{REF})}} \quad (1)$$

wherer_{PS} and r_{REF} denote the reaction rates of fluorescent detection probe with $^1O_2$ generated from PS and REF, respectively.T and T_{REF} are the corresponding optical transmittances of PS and REF at the irradiation wavelength.

The PS molecules may aggregate in aqueous which lowers their quantum yield [11] and poses problems in clinical applications. Nanoparticles when combined with PSs can overcome this problem as well as they can improve the efficiency of the $^1O_2$ formation [12–14]. The PS can either be encapsulated within a matrix or adsorbed or conjugated onto the surface of the nanoparticle. Importantly, nanoparticles can also be targeted to specific cells and/or cell organelle. It is therefore worthwhile to explore the effect of nanoparticles on the generation of $^1O_2$ from the nanoparticle-conjugated PS molecules, especially since only a few reports in the literature [14–16] comment on the SOQY in a nanoparticle-PS system. For example, gold nanoparticles conjugated with photosensitizer, Ce6, showed a 1.6-fold increase of the singlet oxygen production [14]. However these authors did not clarify how the enhancement factor was estimated and how it can be compared with theoretical results. To the best of our knowledge,

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there were no reports that explore the factors affecting the singlet quantum yield of the nano-based system, which is the preliminary objective of our current work.

Herein we discuss $^1O_2$ generation from nanoparticle-PS conjugates using metal (Au) and dielectric (CeF_3) nanoparticle in water. Clinically approved second generation PDT drugs [17–19] Verteporfin (VP) and Rose Bengal (RB) were selected as PSs. The efficiency of the conjugates was investigated based on the SOQY value at different sensitization wavelengths. To explain our results, we have quantified the electric field enhancement around the nanoparticles which contributes to the efficiency of $^1O_2$ generation.

2. Experimental section

2.1. Chemicals

Verteporfin (VP) (catalog No. SML0534), Protoporphyrin IX (PpIX) (catalog No. P8293), Rose Bengal (RB) (catalog No. 330000), Dimethyl sulfoxide (DMSO) (catalog No. D2650), cerium chloride heptahydrate ($CeCl_3{\cdot}7H_2O$) (catalog No.228931), ammonium fluoride (NH_4F) (catalog No. 338869), methanol (catalog No. 322415), Gold(III)chloride hydrate (catalog No. 254169) and polyethylenimine (PEI) (catalog No. 764582) were purchased from Sigma Aldrich, Australia and were used without further purification. SOSG (catalog No. S-36002) was purchased from Invitrogen, USA. The stock solution of VP (3 mM) and Protoporphyrin IX (PpIX) (3.5 mM) was prepared by dissolving 2 mg/ml in DMSO. Both stock solutions were kept in dark below 4 °C. The stock solution of RB (1 mM) was prepared by dissolving 10.5 mg in 10 ml of water and is kept in dark condition to avoid photobleaching. The stock solution of SOSG (500 μM) was prepared by dissolving it in 330 μl methanol. The stock solution of SOSG was kept frozen under dark conditions.

2.2. Synthesis of CeF_3 and Au nanoparticles

CeF_3 nanoparticles were prepared using a simple co-precipitation method according to our previous report [20,21]. After 5 h reaction time the particles were washed several times and dried. A stock solution of NPs was prepared by adding 1 mg of CeF_3 nanoparticles to 1 ml of deionized water (5 mM). Au nanoparticles were prepared at room temperature using poly (ethylenimine)) (PEI) as the reductant of hydrogen tetrachloroaurate ($HAuCl_4$). After 4 h reaction time the solution turned to deep red indicating the formation of Au nanoparticles. The particles were washed several times and dispersed in water.

2.3. Formation of nanoparticle-photosensitizer conjugates

To conjugate CeF_3 with the VP (conjugate A1), 500 μM of CeF_3 and 0.5 μM of VP were mixed at room temperature for 6 h at 200 rpm using a mixer rotator. After 18 h, the mixture was centrifuged at 15,000 rpm for 20 min. The supernatant was removed and washed twice. The same procedure was repeated to conjugate the same amount of CeF_3 with different concentrations of VP (1 μM (conjugate A2) and 1.5 μM (conjugate A3)). The concentration of VP in each conjugate was calculated by comparing the absorption peaks of CeF3 and VP in the conjugate with the absorption peak of pure CeF3 and pure VP with known concentration. The detailed calculation was reported in our previous paper [20]. The final concentration of VP in conjugates A1–A3 are 0.11, 0.41 and 0.9 μM respectively.

To conjugate Au with VP (conjugate B1) through electrostatic binding, 500 μl of Au stock solution (33 μM) was added to 0.5 μM of VP and mixed for 2 min using a mixer rotator. After 24 h, the mixture was centrifuged at 13,000 rpm for 10 min. The supernatant was removed and washed twice. This procedure was repeated for conjugating same amount of Au with 1.0 μM (conjugate B2) and 1.5 μM (conjugated B3) of VP. The final concentration of VP in these conjugates B1–B3 are 0.238, 0.585 and 0.879 μM respectively.

To conjugate Au with RB through covalent binding (conjugate C1), first Au was attached to Thiol-PEG-NH_2(SH-PEG-NH_2)·To achieve this, 5 ml of Au was added 300 μl of 0.88 mM SH-PEG-NH_2, and stirred for 3 h. From this mixture 600 μl was taken, centrifuged and added to 500 μl of MES buffer. A 20 μl of RB from stock was added to 3 ml of MES buffer and 5 mg of EDC and kept it to react for 20 min. After that, PEGylated Au in MES buffer and above RB suspension were mixed together and stirred for 3 h to carry out the conjugation reaction. After 3 h, the conjugate was centrifuged for 8 min at 3500 rpm, washed and 3 ml of water was added. The same procedure was repeated by adding 30 μl (conjugate C2) and 40 μl (conjugate C3) of RB. The final concentrations of RB in the three conjugates were 0.13, 0.36, 0.42 μM.

2.4. Nanoparticle characterization

The size and shape of the nanoparticles were obtained from the transmission electron microscopy (TEM) image taken by PHILIPS CM10 system. The TEM samples were prepared by putting 10 μl of nanoparticle colloid on to 300-mesh copper grid. The size analysis was carried out by taking more than 200 nanoparticles from the TEM image by using an image analyzing software, ImageJ. The colloidal stability (zeta potential) of the nanoparticle is measured using a Zetasizer Nano ZS from Malvern Instruments.

2.5. Absorption measurements

All absorption spectra were measured using a Cary UV/VIS/NIR absorption dual beam spectrophotometer using a pair of 1 cm path length quartz cuvettes.

2.6. Fluorescence measurements

All fluorescence measurements were carried out on a Cary Eclipse Fluorescence spectrophotometer, equipped with Xenon flash lamp as the excitation source. The slit width of 5 nm was used for both excitation and emission. A quartz cuvette was used in all the measurements.

2.7. Sensitization source

Sensitization was performed by using a 365 nm high power LED (at the irradiance of 2.4 mW/cm^2) and 532 nm diode pumped solid state laser (at the irradiance of 119 mW/cm^2). The sensitization time was 10 min and 5 min respectively.

2.8. Monitoring of singlet oxygen using SOSG as probe

For measuring singlet oxygen generation we used singlet oxygen sensor green (SOSG) as the detection probe. 4 μM of SOSG was added to 2 ml of conjugates as well as in the control samples (photosensitizer, water and nanoparticles only). The above mixtures were placed in quartz cuvettes. Fluorescence (500–600 nm) of SOSG was measured before and after irradiation at 488 nm excitation in the Cary Eclipse spectrometer.

3. Results and discussion

In this work, the CeF_3 nanoparticles of 10 nm diameter (the TEM image is shown in Supplementary Fig. S1) were conjugated to three different concentrations of VP while keeping the concentration of