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Bovine serum albumin-capped gold nanoclusters conjugating with methylene blue for efficient $^1O_2$ generation via energy transfer

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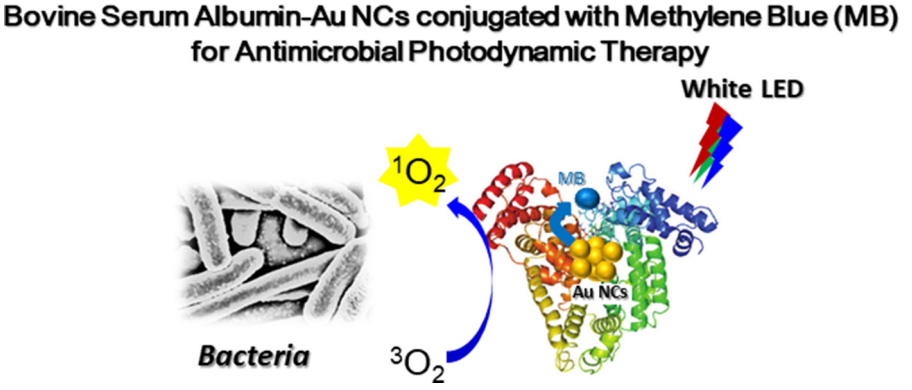
GRAPHICAL ABSTRACT



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ABSTRACT

Bovine serum albumin (BSA)-capped gold nanoclusters (BSA-Au NCs) are attractive photosensitizers for efficient singlet oxygen $^1O_2$ generation owing to their high-water solubility, low toxicity, and the broad absorption from UV to visible wavelengths, and the long lifetime of the electronic excitations (of the order of microseconds). However, the $^1O_2$ generation efficiency of BSA-Au NCs is relatively low. In the present study, a conjugate of BSA-Au NCs and methylene blue (MB) (BSA-Au NC-MB conjugate) has been developed to improve $^1O_2$ generation for antimicrobial photodynamic therapy (aPDT). The BSA-Au NC-MB conjugate demonstrated enhanced $^1O_2$ generation compared to the case of BSA-Au NCs and effective aPDT ability under white-light LED illumination for only 1 min due to the resonance energy transfer from the Au NCs to the MB in the conjugate. To the best of my knowledge, this is first report of Au NCs on the resonance energy transfer application for efficient $^1O_2$ generation. Therefore, the BSA-Au NC-MB conjugate is a novel photosensitizer for $^1O_2$ generation that shows great potential for aPDT, and the present study also develops a very simple strategy to fabricate albumin-based nanoparticles for PDT.

1. Introduction

Over the past 10 years, gold nanoclusters (Au NCs) with sizes less than 2 nm haven been recognized as a new substance possessing unique crystal structures and physico-chemical properties such

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as electronic, magnetic, optical, and chemical properties, which differ from those of plasmonic gold nanoparticles with sizes of more than 3 nm [1–3]. The structures and properties of Au NCs depend on the size at the atomic level and on the surface ligand species, and hence atomically controlled synthetic methods for producing Au NCs in solution have been proposed so far [4–14]. Owing to their attractive nature, researchers have reported various applications of Au NCs including catalysis, fluorescence, sensing, electronics, bio-imaging, biomedical assay, and therapy [15–21].

Reactive oxygen species are chemically reactive molecules including singlet oxygen ($^1O_2$) or superoxide (O_2^-). The chemical properties of $^1O_2$ differ significantly from those of ground-state triplet oxygen ($^3O_2$), and $^1O_2$ readily reacts with a wide range of biological and organic materials which leads to their alteration and degradation. The generation of highly reactive $^1O_2$ is of major importance for a variety of applications such as photodynamic therapy (PDT), water treatment, and catalytic oxidation [22–26]. Photosensitizers are essential for $^1O_2$ production; an excited photosensitizer (typically, organic dye such as porphyrin) transfers energy to $^3O_2$ giving rise to $^1O_2$. As a result, photosensitizers typically have high extinction coefficients, triplet states of appropriate energies to allow for efficient energy transfer to ground-state oxygen, and high triplet-state yields with long triplet-state lifetimes [27].

Recently, Au NCs have been utilized as photosensitizers for generation of $^1O_2$ [25,28–33]. In particular, bovine serum albumin (BSA)-capped Au NCs (BSA-Au NCs) have attracted much attention as photosensitizers for efficient $^1O_2$ generation [34–36], owing to their high-water solubility, low toxicity, the broad absorption from UV to visible wavelengths, and the long lifetime of the electronic excitations (of the order of microseconds). Previously, we have reported the ligand effect on $^1O_2$ generation in biomolecule-protected Au_{25} NCs with different thiolate ligands, Au_{25}(glutathione)$_{18}$ NCs and BSA–Au_{25} NCs; the $^1O_2$ generation efficiency of BSA-Au_{25} NCs was higher than that of Au_{25}(glutathione)$_{18}$ NCs [36].

Methylene blue (MB) is a widely used photosensitizer for PDT applications because of the high quantum yield of $^1O_2$ generation ($\Phi_T \sim 0.52$) via excitation in the therapeutic window (600–900 nm) [37–39]. However, the clinical use of MB has been hampered by its poor stability; MB in the biological environment is usually converted by accepting electrons from NADH/NADPH, and the formed colorless leukomethylene blue has negligible photodynamic activity [40,41]. To resolve this issue, several studies have demonstrated the utility of MB-encapsulated nanoparticle-based PDT, which employs biocompatible nanoparticle matrices [42–46]. As a strategy to achieve MB-encapsulated nanoparticle-based PDT, photosensitizer-conjugated serum albumins with MB have been reported due to the advantages of serum albumins such as biocompatibility and good stability [47]. However, the generated $^1O_2$ is dramatically quenched by the surrounding serum albumins, since BSA is a quencher of $^1O_2$ [48,49]. Thus, it is a challenge to develop a photosensitizer-conjugated serum albumin system for enhanced $^1O_2$ generation.

In this study, we have fabricated a conjugated system of BSA-Au NCs and MB (BSA-AuNCs-MB conjugate). This conjugate showed a $^1O_2$ generation efficiency as high as that obtained by free MB under white-light LED irradiation. The enhanced $^1O_2$ production by the BSA-AuNCs-MB conjugate was explained by resonance energy transfer (RET) from the Au NCs to the MB in the conjugate, where the Au NCs act as a donor, the MB dyes act as an acceptor, and the BSA work as a bridge to form Au NCs-MB conjugates for the RET. Finally, the conjugate had a significant antimicrobial photodynamic therapy (aPDT) effect on Streptococcus mutans under white-light LED illumination for only 1 min.

2. Materials and methods

2.1. Chemicals

All the chemicals were used as received without further purification. Tetrachloroauric(III) acid ($HAuCl_4 \cdot 3H_2O$, 99.99%), methotrexate (MTX, 98%), dimethylformamide (DMF, 99.5%), bovine serum albumin (BSA, >95%), methanol (99.9%), methylene blue (MB, 98.5%), and heavy water (D_2O, 99.9%) were purchased from Wako Pure Chemical Industries Ltd. Nanopure water (resistivity: 18.2 MΩ·cm) was obtained using a Barnstead NANO pure DI water system. Human serum is purchased from Funakoshi Co., Ltd.

2.2. Synthesis

2.2.1. BSA-Au NCs

The synthesis of BSA-Au NCs was performed according to the method described in the literature [9]. Typically, 5 mL of $HAuCl_4$ solution (10 mM) was thoroughly mixed with 5 mL of BSA solution (50 mg/mL, 0.76 mM) under vigorous stirring for 10 min. The pH of the mixed solution was then adjusted to about 11 by adding 1 M NaOH solution (1 mL). After reaction at 37 °C for 8 h, a clear brown solution of Au NCs was obtained. The solution of BSA-Au NCs was thoroughly dialyzed for two days with water. After that, the solution of BSA-Au NCs was filtered through a 0.22 μm membrane filter.

2.2.2. BSA-Au NCs-MB conjugates

BSA-Au NCs-MB conjugates were prepared through the interaction between BSA and MB. A 1 mM MB solution and a 1 mM BSA-Au NC solution were prepared as stock solutions. The MB solution was mixed with the BSA-Au NC solution in mole ratios of 3:1, 1:1, 0.3:1, and 0.1:1 (Au NC: MB). Herein, we used Au NCs with 25 gold atoms (i.e., BSA-Au_{25} NCs) [9]. The resultant solution was stirred at 200 rpm for 2 h using a magnetic stirrer. After that, the solution was purified with a centrifugal ultrafiltration tube (Millipore, 3 KD) to discard the free MB. After centrifuged ultrafiltration, the filtrate solution (<MW 3000) did not contain MB, even at the ratio of 0.1:1 (i.e., colorless filtrate solution), indicating the binding of MB into the BSA-Au NCs.

2.3. Detection of $^1O_2$

The $^1O_2$ generation by photo-excited BSA-Au NCs-MB conjugates was evaluated with a chemical trap $^1O_2$ probe, MTX [50]. It is known that $^1O_2$ can selectively oxidize non-fluorescent MTX to form a fluorescent species. Typically, a 10 mM stock solution of MTX in DMF was prepared, and a 2 mL aqueous solution (D_2O) of the conjugates was then added to obtain a final concentration of MTX of 20 μM. The solutions were then irradiated with a white light-emitting diode (LED) (15 mW, 80 mW/cm^2 at 450 nm, SPF-D2, shodensha, Osaka, Japan) to detect $^1O_2$ generated by the conjugates.

2.4. Measurements

UV–Vis absorption spectroscopy and steady-state fluorescence spectroscopy measurements were conducted using JASCO V-670 and FP-6300 instruments, respectively. All the measurements were performed using 1 cm cuvettes at room temperature. Fluorescence lifetime of BSA-Au NCs-MB, BSA-Au NCs, and MB was measured by time-correlated single photon counting using an LED at 470 nm with a Quantaurus-Tau fluorescence lifetime measurement system (C11367-03, Hamamatsu Photonics Co.). Time-resolved emission