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A near-infrared BSA coated DNA-AgNCs for cellular imaging

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ABSTRACT

Near-infrared silver nanoclusters, have potential applications in the field of biosensing and biological imaging. However, less stability of most DNA-AgNCs limits their application. To obtain stable near-infrared fluorescence DNA-AgNCs for biological imaging, a new kind of near-infrared fluorescent DNA-Ag nanoclusters was constructed using the C3A rich aptamer as a synthesis template, GAG as the enhancer. In particular, a new DNA-AgNCs-Trp@BSA was obtained based on the self-assembly of bovine serum albumin (BSA) and tryptophan loaded DNA-AgNCs by hydrophobic interaction. This self-assembly method can be used to stabilize DNAn-Ag (n = 1–3) nanoclusters. Hence, the near-infrared fluorescence DNA-AgNCs-Trp@BSA was applied in cellular imaging of HepG-2 cells.

1. Introduction

The development of fluorescence probes has made it one of the most powerful tools for the study of cellular processes [1,2]. In particular, near-infrared fluorescent probes have attracted increasing attention because near-infrared light can reach the maximum penetration depth and carry on image of deep tissue [3,4]. Near-infrared metal nanoclusters, especially silver nanoclusters, have attracted great attention for their virtues of unique fluorescence properties and potential applications in the field of biosensing and biological imaging [5–7]. Many ligands have been used to build silver nanoclusters, such as dendrimers [8,9], peptides [10], polymers [11], microgels [12] and oligonucleotides. Among these templates, the oligonucleotides have been used in the construction of silver nanoclusters [13,14]. Silver ions could bind to N3 in pyrimidines and N7 in purines of DNA [15]. The fluorescence emission of DNA-mediated silver nanoclusters can be tuned throughout the visible and near-IR range by modulating the sequence of DNA template [16,17]. Compared with dyes and dots, the virtues of facile synthesis, good biocompatibility and low toxicity make DNA-templated silver nanoclusters better candidates in biological imaging [18]. For example, DNA-templated silver nanoclusters functionalized by AS1411, an antiproliferative G-rich phosphodiester oligonucleotide, were reported as cancer cells imaging agents [19–21]. However, the emission of most DNA-AgNCs could be quenched by metal ions (such as Cu^{2+}) or compounds with π-conjugated system due to intercalation [22,23]. The short time stability of most DNA-AgNCs is a critical issue that may limit their usefulness in biological application. Therefore, a stable, near-infrared fluorescence DNA-AgNCs are needed for biological imaging [24]. In this report, a new kind of near-infrared DNA-Ag nanoclusters was constructed using the sequence 5′-CCCACCCACCCTCCCA-3′ as a template to synthesize the silver clusters and 5′-ACAACAGAGGAG-3′ as the enhancer. To improve the stability of DNA-Ag nanoclusters, tryptophan containing DNA-Ag nanoclusters (DNA-AgNCs-Trp) was obtained, which can form new stable DNA-AgNCs-Trp@BSA based on the self-assembly of bovine serum albumin (BSA) and tryptophan by hydrophobic interaction. Furthermore, the DNA-AgNCs-Trp@BSA was applied in biological imaging of HepG-2 cancer cells.

2. Materials and methods

2.1. Chemicals and reagents

The aqueous solutions were prepared with ultrapure water. HepG-2 cancer cells were supplied by College of Pharmacy, Jiangsu University. All DNA were purchased from Sangon Biotech. Silver nitrate (99%, A.C.S. reagent), sodium silicate, citric acid, sodium hydroxide, $NaBH_4$ (power, 96%), tryptophan and bovine serum albumin (BSA) were obtained from the Sinopharm Chemical Reagent Co. Ltd. Graphene oxide (GO) was purchased from Xianfeng nanometer Mster Technology Ltd. Dialysis membrane (MWCO: 3500; diameter: 29 mm; length: 1 m) was purchased from Source Leaf Biological Technology Co. Ltd.

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2.2. Synthesis of DNA-AgNPs

Silver nanoclusters were prepared by combining DNA (5′-CCCACCCACCCTCCCA-ACAACAGAGGAG-3′) (64 μL, 100 μM) and Ag^+ solution (20 μL, 10 mM) in a 10 mM citrate buffer (876 μL) at pH = 5.0. The final concentration of $AgNO_3$ was 200 μM. Then, the mixture solution was heated to 71 °C, and maintained at this temperature for 3 min and slowly cooled to the room temperature. An aqueous solution of $NaBH_4$ (40 μL, 10 mM) was then added to give a final concentration of 400 μM and vigorously shaken for 1 min. Fluorescence DNA-AgNCs were obtained after the above solution was stored in the dark for 30 h at 4 °C and were purified with semipermeable membrane.

2.3. Synthesis of DNA-AgNCs-Trp@BSA

DNA and tryptophan were mixed in a 10 mM citrate buffer at pH = 5.0. Then, Ag^+ solution was added into the mixture to give a concentration ratio of Ag^+:oligonucleotide = 30:1. Then, the solution was heated to 71 °C, and maintained at this temperature for 3 min and was slowly cooled to the room temperature. An aqueous solution of $NaBH_4$ was then added to give a final concentration of 400 μM and vigorously shaken for 1 min before kept in the dark for 30 h at 4 °C. Finally, aqueous solution of BSA was added and shaken for 30 min in dark resulting DNA-AgNCs-Trp@BSA. DNA-AgNCs-Trp@BSA were purified with semipermeable membrane.

2.4. Biosilicification treatments to DNA-AgNCs

Silicic acid (30 mM) was prepared fresh before use as follow [25]: 5.3 μL of sodium silicate was diluted in 1 mL of PBS (pH 7.4) and 20 μL of 1.25 M HCl was added to adjust pH of the mixture to 7.5–8.0. To obtain silicified DNA-AgNCs, 30 mM silicic acid in PBS and DNA-AgNCs were mixed at a ratio of 1:9 and stirred for 5 min. The pH of the mixture was adjusted to 5.5–6.5 with HCl to initiate silicification and was reacted for 15–30 min. Silicification was terminated by adjusting the pH to 7.0.

2.5. GO stabilized DNA-AgNCs

DNA and GO (0.05 mg/mL, 100 μL) were mixed in a 10 mM citrate buffer at pH = 5.0 with ultrasonic treatment for 1 h. Ag^+ solution was added into the mixture to give a concentration ratio of Ag^+:oligonucleotide = 30:1. Then, the solution was heated to 71 °C, and maintained at this temperature for 3 min and was slowly cooled to the room temperature. Finally, an aqueous solution of $NaBH_4$ was added to give concentration ratio of BH_4^-:Ag^+ = 2:1 and vigorously shaken for 1 min and was left in dark for 30 h at 4 °C to form GO stabilized DNA-AgNCs.

2.6. Cell imaging

HepG-2 cancer cells (hepatoma cancer cell) was inoculated into culture plate with 2.4×10^4 cells in each well and incubated for 24 h. DNA-AgNCs and DNA-AgNCs-Trp@BSA were purified with semipermeable membrane and diluted to an appropriate concentration with culture solution respectively, and then incubated with HepG-2 cancer cells for 4 h at 37 °C. The medium was washed three times with PBS buffer to remove the nanoclusters in medium before detecting with Nikon I-E 2000 microscope. The excitation wavelength of DNA-AgNCs and DNA-AgNCs-Trp@BSA was 740 nm.

2.7. ICP-MS analysis

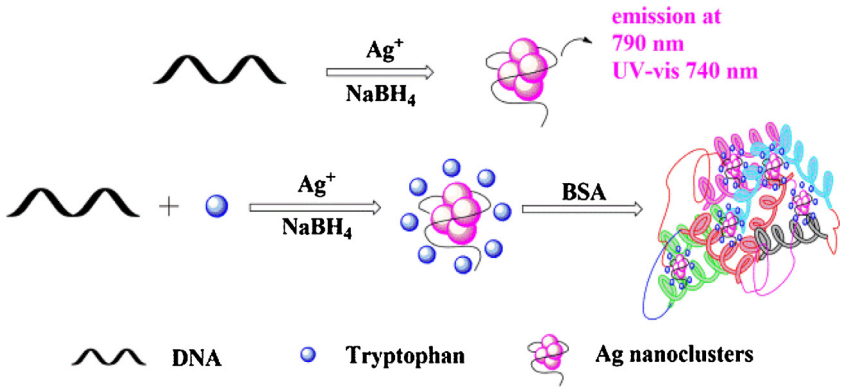
After 4 h purification with semipermeable membrane, DNA-AgNCs (6.4 μM, 200 μL) was digested with 50% nitric acid (HNO_3). Samples were dried at 200 °C and residues were dissolved with 2 mL of 1% HNO_3 and tested using ICP-MS (Agilent 7700) after 10-fold dilution with 1% HNO_3.

3. Results and discussions

3.1. Characterization of DNA-AgNCs and DNA-AgNCs-Trp@BSA

A new kind of DNA-mediated silver nanoclusters with near-infrared fluorescence was prepared using an oligonucleotide as template, with 5′CCCACCCACCCTCCCA3′ as a scaffold and 5′ACAACAGAGGAG3′ as a functional site. For the formation of DNA-AgNCs, the silver salt is mixed with DNA solution, then Ag^+ ions bind to N3 in pyrimidines and N7 in purines of DNA forming DNA-$(Ag)_n^{n+}$ [15]. When $NaBH_4$ is added, DNA-$(Ag)_n^{n+}$ was reduced resulting fluorescence DNA-Ag clusters (DNA-AgNCs). To improve the fluorescent stability of DNA-AgNCs, which would be highly desirable, tryptophan (Trp) and bovine serum albumin (BSA) were introduced in synthetic process. A new DNA-AgNCs-Trp@BSA was formed based on the self-assembly bovine serum albumin (BSA) and tryptophan by hydrophobic interaction (Scheme 1). Detailed verification of DNA-AgNCs and DNA-AgNCs-Trp@BSA morphology is performed by TEM analysis (Fig. 1). The sizes of DNA-AgNCs are in the range of 2–4 nm (Fig. S6A). Fig. 1B shows that Ag nanoclusters are coated with BSA and the sizes of DNA-AgNCs-Trp@BSA are in average of 60 nm (Fig. S6B).

The maximum emission of DNA-AgNCs was at 790 nm when excited at 740 nm. The UV absorption bands of DNA-AgNCs were seen at 400 nm, 550 nm and 740 nm corresponding to the distinguishing absorption of silver clusters (Fig. 2). The UV–vis spectra of DNA, DNA-AgNCs, DNA-AgNCs-Trp and DNA-AgNCs-Trp@BSA were also recorded as indicated (Fig. S1). As known, a peak at 260 nm is typically the absorption of DNA. For proteins and tryp-



Scheme 1. Formation of near-infrared fluorescent DNA-AgNCs-Trp@BSA.