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Novel and remarkable enhanced-fluorescence system based on gold nanoclusters for detection of tetracycline

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ABSTRACT

Tetracycline and Eu^{3+}, while coexisting, usually appear as a complex by chelating. This complex shows low fluorescence intensity, leading to its limitation of analytical goals. Gold nanoclusters (AuNCs), emerging as novel nano-material, are attracting increasing attentions in multiple fields. Herein, gold nanoclusters first function as a fluorescence-enhanced reagent rather than a conventional fluorescent-probe, and a dramatic enhanced-fluorescence system was built based on Eu^{3+}–Tetracycline complex (EuTC) by introducing gold nanoclusters. Simultaneously, three types of gold nanoclusters were employed for exploring various conditions likely affecting the system, which demonstrate that no other gold nanoclusters than DNA-templated gold nanoclusters enormously caused fluorescence-enhancement of EuTC. Moreover, this enhanced-fluorescence system permitted available detection of tetracycline (TC) in a linear range of 0.01–5 μM, with a detection limit of 4 nM at a signal-to-noise ratio of 3. Significantly, the practicality of this method for detection of TC in human urine and milk samples was validated, demonstrating its advantages of simplicity, sensitivity and low cost. Interestingly, this system described here is probably promising for kinds of applications based on its dramatically enhanced-fluorescence.

1. Introduction

Metal-enhanced fluorescence (MEF), usually generated by the interactions of fluorophores with metal [1], has been widely employed as a basic mechanism for medical diagnostics, imaging, and biosensors due to its enhanced-fluorescence signal, decreased lifetime, and improved photostability and sensitivity. To achieve MEF, metallic nanomaterials, especially gold or silver, serve as a critical factor for the fluorescence enhancement of green fluorescent protein, organic fluorophores, quantum dots, upconversion nanocrystals, and lanthanide chelates [2,3]. However, development of enhancing fluorescence by exploring new nanomaterials is still lacking.

Complexes, formed by rare earth metal and organic ligands, typically exhibit superior light absorption, fluorescent characteristic and other advantages including avoiding background interference of organisms and exerting long fluorescence lifetime, large Stokes Shift and narrow emission band [4–6]. As been well known, TC, performing its antibacterial action by interactions with chelate metal [7], functions as one member of antibiotics. In addition, TC and their derivatives have been widely exploited for analytical purposes [8]. As reported in recent years, TC can form complex with the rare metals such as Eu^{3+} through the formation of an organic chelate. In particular, EuTC has served as a sensitive enhanced-fluorescence probe for determining double-stranded and single-stranded DNA [9–12].

Noble metal nanoclusters (NCs), existing in the size of less than 10 nm, usually consist of several to hundred atoms, with properties regulated by their subnanometer dimensions [13–16]. Particularly, gold nanoclusters have been used for different kinds of applications. Up to date, AuNCs are synthesized by two major ways. One way is based on the template-assisted synthesis with polymers [17] and biomolecules (e.g., proteins [18–20] and DNA [21,22] commonly as templates. AuNCs are generally produced by another way of monolayer protection in the presence of molecules with thiol ligands [23–26]. Compared with quantum dots (QDs), AuNCs exist in ultra-small size with low toxicity. As a new type of fluorescent material, unique characteristics of AuNCs have recently attracted growing attentions, potentiating it as a satisfactory candidate for biosensing, catalysis, and imaging [13,16,27,28].

Nanoparticles have been reported to enhance the fluorescence of EuTC by MEF [1], thus we asked whether this complex can be enhanced by nanoclusters, since they are one special type of nanoparticles. In this study, we first developed a novel dramatic enhanced-fluorescence system based on Eu^{3+}-Tetracycline complex by introducing DNA-templated gold nanoclusters. Subsequently, we tested kind of aspects possibly affecting to construct

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an enhanced-fluorescence system by employing three types of AuNCs. Compared with AuNCs@BSA and AuNCs@His, only DNA-templated gold nanoclusters enormously caused fluorescence-enhancement of EuTC, suggesting EuTC with DNA-templated gold nanoclusters may broaden potential ways to design more sensitive assays and fluorescent probes. In addition, this system has been applied to assay TC while Eu^{3+}-AuNCs@DNA_{C12} considered as a base. Importantly, its practicality of this method was validated by detections of TC in human urine and milk samples, and satisfactory results obtained suggested this strategy may broaden avenues for detection of TC.

2. Experimental

2.1. Materials and reagents

Bovine serum albumin (BSA), amino acids and all the DNA sequence were purchased from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). Hydrogen tetrachloroaurate trihydrate ($HAuCl_4$), europium oxide (Eu_2O_3) and all the metal ions were obtained from Sigma-Aldrich (Milwaukee, WI). Sodium tetraborate was purchased from Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China). Ultrapure water, 18.25 MΩ cm, produced with an Aquapro AWL-0502-P ultrapure water system (Chongqing, China) was employed for all procedures.

2.2. Apparatus

All fluorescence measurements were performed on a Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan) with excitation slit set at 5 nm band pass and emission at 5 nm band pass in 1 cm × 1 cm quartz cells. The high-resolution transmission electron microscopy (HRTEM) images were obtained using a TECNAI G2 F20 microscope (FEI, America) at 200 KV. Photographs were taken with an Olympus E-510 digital camera (Tokyo, Japan). A Fangzhong pHS-3C digital pH meter (Chengdu, China) was used to measure pH values of the aqueous solutions and a vortex mixer QL-901 (Haimen, China) was used to blend the solution. The thermostatic water bath (DF-101s) was bought from Gongyi Experimental Instruments Factory (Henan, China).

2.3. Preparation of three kinds of AuNCs

Three types of AuNCs were prepared as follows: the AuNCs@BSA was prepared by a reported method [18]. Briefly, $HAuCl_4$ solution (10 mM, 5 mL) was mixed with BSA solution (50 mg mL^{-1}, 5 mL) under vigorous stirring at 37 °C. Two minutes later, NaOH solution (1.0 M, 0.5 mL) was added, and the mixture was incubated at 37 °C for 12 h. Similarly, the AuNCs@DNA_{C12} were prepared as previously reported [21]. The synthesis reaction was performed by 100 μM $HAuCl_4$, 25 μM DNA_{C12} and 50 mM citrate, and citrate served as not only a buffer (pH=3) but also a reducing agent. The mixture was incubated overnight at room temperature. Furthermore, the AuNCs@His was synthesized by using a biomineralized approach [29]. Typically, an aqueous solution of $HAuCl_4$ (8 mL, 10 mM) was mixed with an aqueous solution of histidine (24 mL, 0.1 M) at room temperature and incubated for 2 h to obtain the desired nanoclusters.

2.4. Construction of the enhanced-fluorescence system

A stock solution of Eu^{3+} was prepared by dissolving Eu_2O_3 with a small amount of nitric acid (0.10 M), and then diluted with ultra water. Then TC was added into diluted Eu^{3+} solution, and the working solution EuTC (2.5×10^{-5} M) was obtained by appropriate dilution. Subsequently, three kinds of AuNCs prepared here were diluted for different times, and further subjected to EuTC respectively to form the enhanced-fluorescence system.

2.5. Detection of TC and interference studies

A stock solution of TC was prepared before use. For detection of TC, 50 μL of buffer solution (sodium tetraborate buffer, pH=9) was mixed with 50 μL Eu^{3+} solution and 10 μL AuNCs@DNA_{C12}, and then 390 μL of different concentration of TC solution was added. After reaction for 5 min at room temperature, the fluorescence intensity of the mixture was measured upon being excited at 375 nm. To investigated the interference of foreign substances, amino acids (Gly, Cys, Ala, Glu, Ser, Val, Lys, Phe, and His), glucose and ascorbic acid were introduced. To evaluated the effects of other metal ions on the fluorescence of EuTC-AuNCs@DNA_{C12}, several metal ions (Na^+, K^+, Ca^{2+}, Cd^{2+}, Pb^{2+}, Ag^+, Cu^{2+}, Co^{2+}, Ba^{2+}, Fe^{3+}, Ni^{2+}, Mg^{2+}, Zn^{2+}, and Hg^{2+}) were added.

2.6. Analysis of TC in urine and milk samples

Human urine sample from one healthy volunteer was collected from Southwest University, Chongqing. For the detection of TC, impurities were precipitated from the urine sample by centrifugation (600 rpm, 10 min). Then, the supernatant was collected into 2 tubes and supplemented with standard TC solutions (2 μM, 4 μM). And the urine samples were filtered through a 0.22 μm membrane and collected in aliquots. Milk samples were collected from Yonghui Supermarket, Chongqing. Briefly, proteins in the milk samples were removed firstly by adding 1% trichloroacetic acid into the milk and sonicating 20 min. After filtering through a 0.22 μm membrane to remove lipids, the supernatant were collected into 3 tubes and spiked with TC solutions (0.5 μM, 3 μM, 6 μM). All the spiked samples described here were further subjected to the detection procedure.

3. Results and discussion

3.1. Characterization of three kinds of AuNCs

To visualize three types of AuNCs, high-resolution transmission electron microscopic (HRTEM) images were obtained. AuNCs@BSA, AuNCs@DNA_{C12} and AuNCs@His displayed different patterns respectively. The majority of AuNCs@BSA are within the size of less than 3 nm (Fig. 1A and D), and AuNCs@DNA_{C12} existing as the size of 1–3 nm were described in Fig. 1B and E, and AuNCs@His is monodispersed with the smallest size (Fig. 1C and F). Additionally, the absorption spectra of these three kinds of AuNCs were recorded (Fig. S1) respectively. Taken together, these data indicated that three kinds of AuNCs have been successfully synthesized [18,21,29].

3.2. Enhanced-fluorescence system built by introducing AuNCs@DNA

Basically, TC coordinates with Eu^{3+} to form a complex under certain conditions, and the fluorescence intensity of this complex could be enhanced by MEF as reported [1]. To broaden its applications, here we tried to pursue another avenue to enhance the fluorescence intensity of EuTC. Subsequently, we tested the possibility to construct an enhanced-fluorescence system by introducing AuNCs to EuTC.

As shown in Fig. 2A, the fluorescence enhanced was about 2-fold of the signal of EuTC while AuNCs@BSA (0.5 mg mL^{-1}) added. Contrastingly, when added into the complex of EuTC, AuNCs@D-NA_{C12} (0.5 mg mL^{-1}) showed a sharp enhanced-fluorescence with