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A novel silver nanocluster in situ synthesized as versatile probe for electrochemiluminescence and electrochemical detection of thrombin by multiple signal amplification strategy

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ABSTRACT

In this work, a novel silver nanoclusters (AgNCs) were in situ synthesized and used as versatile electrochemiluminescence (ECL) and electrochemical (EL) signal probes for thrombin detection by using DNAzyme-assisted target recycling and hybridization chain reaction (HCR) multiple amplification strategy. The presence of target thrombin firstly opened the hairpin DNA, followed by DNAzyme-catalytic recycling cleavage of excess substrates, which could generate large number of substrate fragments (s1). Then these s1 fragments were captured by SH−DNA on the Au nanoparticle-modified electrode, which further triggered the subsequent HCR of the hairpin DNA probes (H1 and H2) to form the long dsDNA. The numerous AgNCs were thus in situ synthesized by incubation the dsDNA template (with cytosine-rich loop)-modified electrode in solution with $AgNO_3$ and sodium borohydride. By integrating the DNAzyme recycling and HCR dual amplification strategy, the amount of AgNCs is dramatically enhanced, leading to substantially amplified ECL and electrochemical signals for sensitive thrombin detection. Importantly, this design introduces the novel AgNCs into versatile ECL and EL bioassays by multiple amplification strategy, thus it is promising to provide a highly sensitive platform for various target biomolecules.

1. Introduction

The trace analysis and quantification of biomolecules such as DNA and protein are closely related to human health as well as biological activities (Fields, 2001; Liu et al., 2009). Thrombin plays crucial roles in various life processes and relates to many diseases, such as, cardiovascular diseases, inflammation reactions, thromboembolic disease, and anticlotting therapeutics (Li et al., 2012; Xu et al., 2015). However, many of the biomarkers are present at an ultralow level in the early stage of diseases. Therefore, developing ultrasensitive detection methods of the biomarkers are critical in clinical diagnosis (Jie et al., 2013).

Electrochemiluminescence (ECL) and electrochemical analysis have remarkable features such as simplicity, rapidity, high sensitivity, easy controllability and low background (Richter, 2004; Miao, 2008), and have been widely employed in bioanalysis (Miao, 2008, Chen et al., 2011; Muzyka, 2014). In particular, quantum dot (QD)-based ECL assays have attracted significant attention in basic research and clinical applications due to its size controlled luminescence, high quantum yield, and stable light emission (Zhao et al., 2015; Jie et al., 2012). However, heavy metals as the essential elements in available high ECL performance semiconductor QDs exhibited the inherent toxicity, which have raised serious health and environmental concerns (Derfus et al., 2004). Thus, it is of great value to develop novel low-toxicity or nontoxic ECL nanomaterials. Gold nanoclusters (Au NCs) and silver nanoclusters (Ag NCs) have attracted substantial research effort owing to their low toxicity, good biocompatibility, and excellent stability (Liu et al., 2010; Shang et al., 2011; Wei et al., 2011; Hesari et al., 2014a, 2014b). In particular, AgNCs as ultrasmall particles containing 2–30 of Ag atoms (Zheng et al., 2007) exhibit dramatically different optical, electronic, and chemical properties compared to nanoparticles or bulk materials (Wilcoxon and Abrams, 2006; Xu and Suslick, 2010). However, the synthesized noble metal nanoclusters were commonly applied in solution-phase ECL, which caused several disadvantages of the high background signal and complicated experimental design compared with noble metal nanocluster-based solid-state ECL. Recently, DNA-stabilized Ag NCs have been applied as multifunctional biolabels (Tao et al., 2014; Liu et al., 2015; Zhang et al., 2015). For example, the photoluminescent Ag NCs-functionalized nanowires were prepared by assembly of DNA-stabilized Ag NCs (Orbach et al., 2013).

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Although more and more methods of AgNCs-based fluorescence detection have been reported, the AgNCs have been seldom applied to ECL and electrochemical analysis. Moreover, the AgNCs with good oxidation-reduction property has promising application in ECL and electrochemical analysis, but the reported DNA-stabilized Ag NCs were almost prepared in homogeneous aqueous solution, which suffered the complicated operation procedure.

On the other hand, to develop highly sensitive biosensors for the early diagnosis of serious diseases, various signal amplification strategies, such as rolling circle amplification (RCA) (Wu et al., 2010), strand displacement amplification (SDA) (Shlyahovsky et al., 2007), and nuclease-assisted multiple amplification strategy (Jie et al., 2017, 2011; Luo et al., 2013; Liu et al., 2013a; Liu et al., 2013bb) have been explored to improve detection capability. Recently, DNAzymes have received great attention owing to their better stability than protein enzymes (Li and Lu, 2000; Zhang et al., 2011). Several DNAzyme probes were used to develop different amplified strategies for DNA detection (Zhao et al., 2013; Sando et al., 2003; Sun et al., 2010). In addition, hybridization chain reaction (HCR) is also one of the most attractive enzyme-free amplification methods (Venkataraman et al., 2007; Peng et al., 2008), and has been applied to nucleic acid and protein sensing assays (Zhu et al., 2013; Liu et al., 2013a; Liu et al., 2013b). However, most of these ECL and electrochemical biosensors by using various amplification strategy have only one signal label, either the type of "ECL" or "electrochemistry", thus the versatile biosensors based on a novel multifunctional nanomaterial probe combined with multiple amplification strategies have remained to be developed.

Herein, taking advantages of the bifunctional AgNCs and multiple amplification strategy, we synthesized the novel AgNCs and used them as versatile ECL and electrochemical signal probes for amplified detection of thrombin by DNAzyme-assisted target recycling and HCR multiple amplification strategies. The target thrombin firstly triggered the DNAzyme-catalytic recycling cleavage of substrates, which led to the first amplification of target. Then the abundant substrate fragments (s1) further induced the HCR of the hairpin DNA probes (H1 and H2) to form the long dsDNA on the electrode (the second amplification). The numerous AgNCs were in situ synthesized on the long dsDNA template-modified electrode, generating substantially amplified ECL and electrochemical signals for sensitive detection of thrombin. This versatile biosensor opened a new way for sensitive detection of various target biomolecules by using the novel AgNCs in situ synthesized.

2. Experimental section

2.1. Synthesis of Au NPs

Gold nanoparticles (Au NPs) were prepared according to the reported method (Frens, 1973). In brief, 2.0 mL of 1.0% $HAuCl_4$ solution was added to 100 mL of boiling water and refluxed for 10 s, then 3.8 mL of sodium citrate (2.0 wt%) was injected into the above solution with vigorous stirring. After boiling for 15 min, the solution turned brilliant ruby red, and the heating was stopped. The stirring was continued for an additional 15 min, then the solution was cooled to room temperature for further use.

2.2. DNA treatment

100 mL of TE buffer solution (pH=7) was prepared as diluent for DNA, the TE buffer was composed of 10 mmol/L Tris-HCl, 1.0 mmol/L EDTA and 12.5 mmol/L $MgCl_2$. DNA was firstly centrifuged at 12,000 rpm for 5 min, then TE buffer was added to the tube with DNA, the DNA concentration was 100 μM, and the DNA was stored at 4 °C for use.

2.3. Procedure for DNAzyme recycle

For target-triggered multiple amplification, the DNAzyme recycle process was performed as follows (Xia et al., 2015). Frist, 1 μM of hairpin DNA was preactivated at 37 °C for 1 h, then 10 μL of different concentrations of thrombin (2 μM) were added and incubated in Tris-HCl binding buffer at 37 °C for 1 h. Subsequently, 5 μL of the substrate (3 μM) and 10 μL of zinc acetate (1 mM) were added to the above-mentioned solution to make a final volume of 50 μL, followed by incubating at 37 °C for 1 h to initiate the cleavage and recycle reactions.

2.4. Fabrication of biosensor

The gold electrodes were polished with 1.0, 0.3 and 0.05 μm alumina powder separately, and rinsed with ultrapure water. Then, the electrodes were further electrochemically cleaned in 0.5 M H_2SO_4 with potential scanning from −0.3 and 1.5 V until a reproducible cyclic voltammogram was obtained, followed by rinsing with ultrapure water and drying with nitrogen. After that, 8 μL of 0.05% PDDA solution was dropped on the pretreated electrodes surface and dried in air. Then the electrodes were immersed in gold colloid solution for 20 min, washed with PBS buffer and dried under a N_2 flow.

Next, 8 μL of SH-DNA (0.5 μM) in buffer (10 mM Tris-HCl, 1 mM EDTA, 10 mM TCEP, 0.1 M NaCl, pH 7.4) was dropped on the modified electrodes at room temperature for 12 h. After being rinsed with ultrapure water and drying with nitrogen, 8 μL of MCH (1 mM) was dripped onto the surface of the electrode for 2 h to eliminate the nonspecific binding effect. Then the thiolated capture DNA functionalized electrodes were incubated with the prepared solution after DNAzyme recycle at 37 °C for 1 h to capture the DNA fragments. After washing with PBS, the modified electrodes were incubated with the mixture of H_1 (1 μM) and H_2 (1 μM) at 37 °C for 2 h, then the electrodes were rinsed with PBS and dried. 8 μL of $AgNO_3$ solution (200 μM) in citrate buffer (20 mM sodium citrate, pH 7.4) was dropped on the electrode surface in dark for 15 min. To prepare AgNCs, 5 μL of fresh $NaBH_4$ solution (500 μM in citrate buffer) was carefully added to the electrode surface with $AgNO_3$ at room temperature in dark for 2 h (Rao et al., 2010). Finally, the electrodes were washed with PBS for electrochemical and ECL measurements.

2.5. Electrochemical detection of thrombin

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed on a CHI 660 E electrochemistry workstation. DPV measurements were carried out in PBS by scanning the potential from 0.0 to 0.5 V with the pulse amplitude of 50 mV, pulse width of 25 ms, and sampling width of 16.7 ms.

2.6. ECL detection of thrombin

The modified electrodes above were in contact with 0.1 M PBS (pH 7.4) containing 0.05 M $K_2S_2O_8$ and 0.1 M KCl and scanned from 0 to −2.0 V. The scanning rate was 100 mV/s. ECL signals related to the thrombin concentrations could be measured.

3. Results and discussion

3.1. ECL behavior of the AgNCs in situ synthesized on the electrode

Fig. S1A showed the ECL–potential curve of the AgNCs in situ synthesized on the electrode. The ECL peak appeared at −1.858 V in the cathodic process, resulting from the reaction of AgNCs with $S_2O_8^{2-}$. The possible ECL mechanisms are as follows.

$$AgNCs + e^- \rightarrow AgNCs^{-\bullet} \quad (1)$$