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AgNPs-3D nanostructure enhanced electrochemiluminescence of CdSe quantum dot coupled with strand displacement amplification for sensitive biosensing of DNA

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HIGHLIGHTS

- AgNPs-3D nanostructure for enhancing ECL signal of CdSe QDs was successfully designed.
- A new dual amplification strategy for detection of DNA by using AgNPs-3D nanostructure coupled with SDA was developed.
- It is for the first time AgNPs-3D nanostructure combining cycling SDA to achieve double amplified detection of DNA.
- The present work provide a promising candidate for ECL biosensor for various target biomolecules.

GRAPHICAL ABSTRACT

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ABSTRACT

A novel strategy using Ag nanoparticles-3D (AgNPs-3D) nanostructure enhanced electrochemiluminescence (ECL) of CdSe quantum dots (QDs) coupled with strand displacement amplification (SDA) for sensitive biosensing of DNA was successfully designed. The prepared CdSe QDs with intense ECL were assembled on the poly (diallyldimethylammonium chloride) (PDDA) graphene oxide (GO) nanocomposites modified electrode, then gold nanoparticles (NPs) as the quenching probe was conjugated to the QDs, ECL signal was efficiently quenched. The target DNA induced cycling SDA and generated a large number of DNA s1. The released DNA s1 could open the hairpin DNA with quenching probe. So the presence of low levels of target DNA can potentially result in a significant enhancement of ECL signal. Furthermore, large number of AgNPs were then in situ reduced in the 3D DNA skeleton on the electrode, which dramaticlly enhanced ECL signal of QDs owing to the excellent electrical conductivity, and the much amplified ECL signal change has a quantitative relation with the target DNA. So by combining the AgNPs-3D nanostructure and cycling SDA to achieve greatly amplified detection of DNA, the promising ECL strategy could provide a highly sensitive platform for various biomolecules and has a good prospect for clinical diagnosis in the future.

1. Introduction

During the past decades, DNA detection has become a hot topic

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for scientists of various subjects, ascribed to its importance in gene sequencing, biomedical research, diagnosis and treatment for virus, and so on [1,2]. Sensitive and rapid detection of the gene plays important roles in early screening of cancers. Various measuring techniques including electrochemistry [3], fluorescence [4], chemiluminescence [5], electrochemiluminescence (ECL) [6], and colorimeter [7] have been implemented to achieve sensitive detection of DNA sequence. Among these techniques, ECL has attracted particular attention and is becoming more recognized for DNA detection due to its high sensitivity, rapidness, simple equipment, excellent reproducibility, and low background noise [8]. The ECL biosensor has been widely applied in immunoassays [9], DNA analysis [10], and clinical diagnosis [11].

QD-based ECL has attracted considerable interest in basic research and clinical applications due to its size controlled luminescence, high quantum yield, and stable light emission [12]. In recent years, an increasing number of semiconductor nanocrystals (NCs) have been exploited as ECL reagents [13,14]. However, the limited ECL efficiency of QDs is one barrier for developing QD-based ECL sensors. To improve the detection sensitivity for practical applications, many kinds of amplification methods have been established, including polymerase chain reaction (PCR) [15], hybridization chain reaction (HCR) [16], rolling circle amplification (RCA) [17], etc. Recently, on account of the advantages of high efficiency, adaptability, and simple operation, strand displacement amplification (SDA) has been widely used for detection of nucleic acid [18]. Subsequent work showed that multiple SDA could be modularly connected into reaction cascades in which a strand released in one reaction could serve as an input in a downstream reaction [19]. Such multistage cascades have enabled the construction of a very wide range of functional biochemical reaction networks including computational Circuits [20] and catalytic signal amplification mechanisms [21]. Although SDA has been applied in some ECL assays [22–24], the assays based on a novel nanostructure enhanced QDs ECL combining with SDA has rarely been reported.

Herein, we report a new dual amplification strategy by using DNA strand displacement cycling reaction coupled with AgNPs-3D nanostructure for sensitive detection of DNA based on ECL of CdSe QDs. The target DNA induced cycling SDA and generated a large number DNA s1. The released DNA s1 could open the hairpin DNA with quenching probe, which resulted in a significant change of ECL signal. Furthermore, AgNPs-3D nanostructure for enhancing ECL signal of CdSe QDs was successfully designed. Large number of AgNPs in the 3D DNA skeleton dramaticlly enhanced ECL signal of CdSe QDs. To our knowledge, we have for the first time realized enhancement of CdSe QDs ECL signal using AgNPs-3D nanostructure and achieved double amplified detection of nucleic acids by combining DNA strand displacement cycling reaction, it is promising to provide a highly sensitive platform for various target biomolecules and has a good prospect for clinical diagnosis in the future.

2. Experimental section

2.1. Preparation of the CdSe QDs

CdSe QDs were prepared according to the method with a slight modification [25]. Briefly, 0.0950 g of sodium borohydride was added to a small flask, then 6 mL of ultrapure water was added. The solution was degassed, refilled with nitrogen. Then 0.0947 g of selenium powder was added rapidly, after the selenium powder disappeared completely, the resulting clear NaHSe was obtained.

The ultrapure water (50.0 mL) was added to a 100 mL flask, degassed with nitrogen for 10 min, then 0.5709 g of $CdCl_2 \cdot 2.5H_2O$ was added, and the solution was degassed under stirring. Then 400 μL of mercaptoacetic acid was added, and the pH was adjusted to 10–11, the solution became clear. The freshly prepared NaHSe solution was added to the flask rapidly, after the mixture was refluxed for 30–40 min to obtain the yellow clear CdSe solution.

2.2. Synthesis of PDDA-functionalized graphene oxide

The PDDA-functionalized graphene oxide was synthesized based on a previous work of our group [26]. First, the graphene oxide was prepared from natural graphite powder by the modified Hummers method [27]. Then the graphene oxides obtained were exfoliated by ultrasonication in water for more than 1 h. At last, water solution of homogeneous graphene oxide (1.0 mg/mL) was obtained.

PVP-capped graphene oxide was prepared according to the reported work [28]. In a typical procedure, 80 mg of PVP was added to 20 mL of 0.25 mg/mL graphene oxide solution, followed by stirring for 30 min. The resulting dispersion was washed, centrifuged three times and dissolved in 5 mL of water.

To obtain PDDA-functionalized graphene oxide (PDDA/GO), 0.1 mL of 20 wt % PDDA was mixed with 16.8 mL of 0.625 M KCl, followed by injecting 4.2 mL of PVP-capped graphene oxide, and the resulting solution was sonicated for 1.5 h. The products were washed and centrifuged three times. Finally, the PDDA/GO was redispersed in 4 mL of water.

2.3. Preparation of gold NPs

Gold NPs were prepared according to the method of reduction of tetrachloroauric acid with trisodium citrate. Briefly, 100 mL of 0.01% $HAuCl_4$ solution was heated to boiling with vigorous stirring, and then 3.0 mL of 1% trisodium citrate solution was added dropwise rapidly. After the color of the solution changed from gray yellow to deep red, the heating source was removed and the resulting colloidal suspension was stirred for an additional 15 min to cool down to room temperature.

2.4. Preparation of the quencher probe gold NPs-hairpin DNA

To form the quencher probe gold NPs-hairpin DNA, 1 mL of gold colloid was firstly centrifuged at 10000 rpm for 10 min, following removal of the supernatant, the precipitate was then redispersed in 100 μL of doubly distilled water. 50 μL of 1.0×10^{-6} M thiol-modified hairpin DNA was added to 100 μL of the purified gold colloid. After shaking gently for 16 h at room temperature, the solution was centrifuged at 10000 rpm for 30 min. The oily precipitate was then washed with 10 mM phosphate buffer two times, recentrifuged, and redispersed in 100 μL of PBS, and stored in a refrigerator (4 °C).

2.5. Electrode modification

For ECL detection, the Au electrodes were polished with alumina slurries (1, 0.3, 0.05 μm), washed with deionized water, and dried with nitrogen gas. After 8 μL of PDDA-GO solution was dropped on the electrode surface and dried naturally at room temperature, 8 μL of CdSe QDs solution was dropped on the electrode and dried.

2.6. DNA reaction system

The reaction system (50 μL) was prepared as follows. First, 10 μL of target DNA at different concentrations, 10 μL of template DNA, 2 μL of dNTPs (10 mM), 1.5 μL of endonuclease (NtBsmAI, 20 U), 1.5 μL of Phi29 (16 U) and 5 μL of Buffer were added and incubated