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Sensitive cancer cell detection based on Au nanoparticles enhanced electrochemiluminescence of CdS nanocrystal film supplemented by magnetic separation

Huai-Rong Zhang, Xing-Hua Xia, Jing-Juan Xu *, Hong-Yuan Chen

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, PR China

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ABSTRACT

We report here a sensitive electrochemiluminescence (ECL) approach for detection of HL-60 cancer cells based on Au nanoparticles (NPs) enhanced ECL of CdS nanocrystal (NC) film supplemented by magnetic separation. In this approach, capture DNA-1 modified CdS NC film was used as ECL emitter. Capture DNA-2, which is labeled with Au NP, is hybridized with the aptamer modified on magnetic beads (MBs) to form MB-Au NP bioconjugates. In the presence of HL-60 cells, the aptamer would conjugate with the glycoprotein at cell surface and Au NPs labeled capture DNA-2 would be released. After magnetic separation, the released Au NPs labeled capture DNA-2 is hybridized with the capture DNA-1 to enhance the ECL signal of CdS NC film. This approach could sensitively detect HL-60 cells with a linear calibration range from 20 to 1.0×10^6 cells/mL.

1. Introduction

In recent years, more and more attention has been paid to the electrochemiluminescence (ECL) of semiconductor nanocrystals (NCs) [1] due to their potential applications in bioanalytical chemistry [2]. Semiconductor nanocrystals (NCs) were used based on two principles, namely, electron transfer [3] and energy transfer [4]. The process of electron transfer can be summarized as the electro-chemically generated species to undergo highly-energetic electron transfer (redox or enzymatic) reactions that emit light from excited states, then the light emission was detected. This principle was used to detect cancer cells [5] through binding the cancer cells to the surface of the electrode in order to hindering the electron transport. This method is easy to operate, but the detection range is limited due to the large volume of the cancer cells. ECL energy transfer, as a powerful technique for detecting changes in the distance between donors and acceptors, has also been exploited for the sensitive detection of molecular interactions [6] with a particular target molecule. Considerable works have been done in this area, for example, energy transfer systems based on opto–magnetic interaction [7], blackbody-like energy scavenging effects [8–11] and ECL resonance energy transfer from semiconductor NCs (S-NCs) to $Ru(bpy)_3^{2+}$ [12,13]. Besides, previous works [14–17] also show that surface plasmons (SP) of Au NPs could induce ECL enhancement of S-NCs. When the S-NCs and Au NPs are at close proximity, ECL emission of S-NCs can be easily quenched through nonradiative energy dissipation in the metal. However, when they are separated at a certain distance, the excitation of Au NP surface plasmon resonance (SPR) can create strong local electric fields that in turn modulate the ECL response of the CdS-NCs. Consequently, the ECL signals are greatly amplified.

In this article, the SPR induced ECL enhancement described above was firstly used in sensitive detection of cancer cells. Different from most of the electrochemical and ECL approaches achieving cell detection via capturing cells on the electrode surface, herein, we used magnetic beads modified with aptamers to capture the HL-60 cancer cells in homogeneous solution. This method will avoid the spatial hindrance effect of the electrode surface and broaden the detection range. It gives a linear calibration range from 20 to 1.0×10^6 cells/mL.

2. Experiments

2.1. Reagent

Labeled DNA oligonucleotides including aptamer DNA (5′-ATCC' AGAGTGACGCAGCATGCCCTAGTTACTACTACTCTTTTTAGCAAACGCCCTC GCTTTGGACACGGTGGCTTAGT-3′-SH), capture DNA-1 (5′-GTCACTCT GGATTTTTTTTTTTTT-3′-SH) and capture DNA-2 (5′-ATCCAGAGTGACT TTTTTTTTTT-3′-SH) were purchased from Shenggong Bioengineering Ltd. Company (Shanghai, China). Bovine serum albumin (BSA) and tri (2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Magnetic beads were purchased from Xi'an GoldMag Nanobiotech

* Corresponding author. Tel.: +86 25 83597294; fax: +86 25 83594862.
E-mail address: xujj@nju.edu.cn (J.-J. Xu).

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Co., Ltd. Tris–HCl buffer (0.1 M) containing 0.1 M NaCl (pH 7.4) was employed for hybridization and preparation of DNA stock solutions. 0.1 M phosphate buffer KH_2PO_4–K_2HPO_4 containing 0.1 M NaCl (pH 7.4, PBS) was used to wash the electrode. Millipore ultrapure water was used throughout the experiment.

2.2. Instruments

The UV–vis absorption spectra were recorded on a Shimadzu UV-3600 UV–vis–NIR photospectrometer (Shimadzu Co.). The electrochemical and ECL emission curves were obtained on a MPI-A multifunctional electrochemical and chemiluminescent analytical system (Xi'an Remax Electronic Science &Technology Co. Ltd., Xi'an, China) with a conventional three-electrode system at room temperature. The electrodes were a glassy carbon electrode (GCE) with 3 mm diameter modified with CdS NC composite film served as working electrode, a Pt wire counter electrode and a saturated calomel reference electrode (SCE). The spectral width of the photomultiplier tube (PMT) was 350–650 nm, and the voltage of the PMT was set at −500 V in the process of detection.

2.3. Preparation of capture-DNA-1 modified CdS NCs on GCE electrode

CdS NCs and CdS NCs modified GCE were synthesized and prepared according to our previous work [14]. The solution of 1×10^{-4} M capture SH-DNA-1 (50 μL) was pretreated by 2 μL TCEP, then the CdS NCs modified GCE was immersed in the capture SH-DNA-1 solution for 24 h at 4 °C in order to assure the fastness of Cd – S bond [18]. The obtained electrode was rinsed with 0.1 M NaCl + 0.1 M Tris–HCl buffer to remove the unspecified capture SH-DNA-1.

2.4. Preparation of MB-Au NP bioconjugate

Au NPs were synthesized according to the previous work [15]. 50 μL of 1×10^{-5} M capture SH-DNA-2 solution pretreated by 2 μL TCEP for 1 h was added into 2 mL colloid Au NPs. The mixed solution was kept for 24 h at 4 °C in order to assure the fastness of Au – S bond. After that, 2 wt.% BSA was used to block the nonspecific active binding sites of the Au NPs.

100 μL of a 1×10^{-4} M aptamer solution pretreated by 2 μL TCEP for 1 h was added into 100 μL gold capped magnetic bead solution. The mixed solution was kept for 24 h at 4 °C, and then the solution was washed with PBS buffer for three times. 2 mL of capture-DNA-2-Au NPs and 100 μL of aptamer-magnetic beads were mixed and incubated at 37 °C for 1 h. The resulting MB-Au NP bioconjugates were separated from the incubation solution and washed with PBS buffer.

2.5. Quantitative determination of HL-60 cells via ECL

After HL-60 cells was centrifugated and washed with PBS, the MB-Au NP bioconjugates were incubated with them for 2 h at 37 °C. After gold-magnetic separation, the CdS NCs/GCE electrode modified with capture-DNA-1 was immersed in the above solution for 1 h at 37 °C to form ds-DNA. The electrode was sequentially washed with PBS to remove the unbinding species. The ECL measurement was performed in 0.1 M PBS buffer (5.0 mL; pH 7.4) containing 0.05 M $K_2S_2O_8$.

3. Results and discussion

3.1. The mechanism of ECL and its enhancement by Au NPs

Fig. 1 shows the sensing principle to HL-60 cancer cell based on Au NPs enhanced ECL of CdS NC film supplemented by magnetic separation. The CdS nanocomposite film modified GCE could generate stable ECL [16] when the potential was scanned in the negative direction from 0 to −1.4 V in 0.1 M PBS buffer (5.0 mL; pH 7.4) containing 0.05 M $K_2S_2O_8$ and 0.05 M KCl. Upon the potential scan with an initial negative direction, the CdS NCs immobilized on the electrode were reduced to $CdS^{\bullet-}$, meanwhile, the coreactant $S_2O_8^{2-}$ was reduced to the strong oxidant $SO_4^{\bullet-}$, then $CdS^{\bullet-}$ could react with $SO_4^{\bullet-}$ to produce the excited states of CdS NCs, which decayed to the ground state with light emitting. Also, we can see that in the presence of $K_2S_2O_8$, CdS NC film generated an ECL peak at ca. −1.20 V.

The ECL intensity shows a small decrease after modified capture DNA-1 (Fig. 2A, curve b), however, when the GCE was hybridized with 10^{-5} M DNA-2-Au NP conjugates, there was almost 5-fold enhancement of the ECL signal (Fig. 2A, curve c). This enhancement can be contributed to the existence of the interactions between electrogenerated excitons in ECL of CdS NCs and ECL-induced SPR of Au NPs. When they are separated at a certain distance, the ECL of CdS NC film could excite the SPR of Au NPs which can create strong local electric fields that in turn modulate the ECL response of CdS NCs by the manner in which the photoexcitation can be converted into luminescence. It is obviously that this enhancement can be used to detect the cancer cells effectively. There is slight change of the peak current after modified with capture DNA-1 which caused by the resistance of the capture DNA-1 (Fig. 2A, curve b′ inset), however, the peak current showed an obvious decrease after hybridized with DNA-2-Au NP conjugates due to the inhibition of assembled capture DNA-2-Au NP conjugates. Fig. 2A, curve c′ also shows a slight negative shift of the reduction potential of coreactant $S_2O_8^{2-}$ on CdS NC film after hybridized with capture DNA-2-Au NPs which could be explained by the negative charge of the capture DNA-2-Au NP conjugates.

The enhancement happened only if there was an interaction between excited luminophores and surface plasmon resonances in metal. However, once Au NPs were closely contact with luminophores, they would be good quenchers due to the Förster Resonance Energy Transfer from CdS NCs to Au NPs. The study shows that the strength of both Förster Resonance Energy Transfer and SPR [19] decreased as the increasing distance between CdS QDs and Au NPs. Förster energy transfer induced quenching is a short-range effect, and it would be weakened with the distance much faster than the SPR field responsible for the enhancement in ECL. In this regard, our group have done lots of works, and 12 nm could induce a considerable enhancement, so there are both 24 bases (1 nm for 3-base) in capture SH-DNA-1 and capture SH-DNA-2 with 8 complementary bases, the distance was about 12 nm after hybridization. Besides, the spectral overlap between energy donor and acceptor also has great influence on ECL enhancement efficiency by Au NPs as well as the distance. We detect the UV spectrum of the Au NPs and BSA-capture-DNA-2-Au NPs (Fig. 2B), there is a slight red shift of the absorption maximum peak of BSA-capture-DNA-2-Au NPs compared to the pure Au NPs, and this could be the result of the change of the Au NP surface charges caused by the capture-DNA-2 and BSA. The ECL spectrum of CdS NCs which shows a broad ECL emission peaked at about ca. 530 nm, and the UV spectrum of the capture-DNA-2-Au conjugates exhibited a maximum absorption at about ca. 530 nm. It is obviously that there is a satisfying overlap.

3.2. Detection of the HL-60 cancer cells

In this work, we designed an effectively method to detect the HL-60 cancer cells with great specificity. We used the capture DNA-1 modified CdS NC film as ECL emitter and capture DNA-2 modified Au NPs which was hybridized with the aptamer modified on magnetic beads (MBs) to form MB-Au NP bioconjugates. After the aptamer conjugated with the glycoprotein at cell surface, the capture DNA-2 modified Au NPs was released from the MB-Au NP bioconjugates and hybridized with the capture DNA-1 to enhance the ECL signal of CdS NC film.

On the basic of this ECL enhancement, the result of detection is quite satisfying. The ΔECL between enhanced ECL intensity and without