Biosensors and Bioelectronics 90 (2017) 251–257

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

DNA tetrahedral scaffolds-based platform for the construction of electrochemiluminescence biosensor

Qiu-Mei Feng, Zhen Zhou, Mei-Xing Li, Wei Zhao*, Jing-Juan Xu*, Hong-Yuan Chen

State Key Laboratory of Analytical Chemistry for Life Science and Collaborative Innovation Center of Chemistry for Life Sciences, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, PR China

ARTICLE INFO

Keywords:
DNA tetrahedral scaffolds
Electrochemiluminescence
Surface plasmon resonance
CdS quantum dots
Au nanoparticles

ABSTRACT

Proximal metallic nanoparticles (NPs) could quench the electrochemiluminescence (ECL) emission of semiconductor quantum dots (QDs) due to Förster energy transfer (FRET), but at a certain distance, the coupling of light-emission with surface plasmon resonance (SPR) result in enhanced ECL. Thus, the modification strategies and distances control between QDs and metallic NPs are critical for the ECL intensity of QDs. In this strategy, a SPR enhanced ECL sensor based on DNA tetrahedral scaffolds modified platform was reported for the detection of telomerase activity. Due to the rigid three-dimensional structure, DNA tetrahedral scaffolds grafting on the electrode surface could accurately modulate the distance between CdS QDs and luminol labelled gold nanoparticles (L-Au NPs), meanwhile provide an enhanced spatial dimension and accessibility for the assembly of multiple L-Au NPs. The ECL intensities of both CdS QDs (−1.25 V vs. SCE) and luminol (+0.33 V vs. SCE) gradually increased along with the formation of multiple L-Au NPs at the vertex of DNA tetrahedral scaffolds induced by telomerase, bringing in a dual-potential ECL analysis. The proposed method showed high sensitivity for the identification of telomerase and was successfully applied for the differentiation of cancer cells from normal cells. This work suggests that DNA tetrahedral scaffolds could serve as an excellent choice for the construction of SPR-ECL system.

1. Introduction

Electrochemiluminescence (ECL) is a process of the luminescence generated by relaxation of exited state molecules that are produced during an electrochemically initiated reaction (Fahnrich et al., 2001; Richter, 2004). In contrast to traditional optical and electrochemical sensors, ECL sensors show unique advantages, such as high sensitivity, low background signal and no need of any external light source (Liu et al., 2015; Shamsi et al., 2016; Yu et al., 2016). Since the first report on the ECL study of semiconductor quantum dots (QDs) by Bard's group in 2002 (Ding et al., 2002), QDs have attracted widespread application in bioanalytical and analytical chemistry because of their excellent optical performance including quantum size-controlled luminescence, symmetrical emission spectra, broad excitation spectra and excellent light stability (Duan et al., 2009; Liu et al., 2007; Miao, 2008; Wang et al., 2016). However, compared with conventional luminescent reagents like luminol or $Ru(bpy)_3^{2+}$, QDs usually suffer from relatively weaker ECL emissions. Thus, the key challenge for the ECL sensor of QDs still lies in the improvement of the sensing performance of ECL luminophores. Aiming at this issue, many strategies have been introduced in the design of QDs based ECL biosensors for signal amplification, such as ECL resonance energy transfer (ECL-RET), in situ activation of QDs, etc. In 2009, our group reported the surface plasmon resonance (SPR) of Au NPs to enhance the ECL emission of CdS QDs. The excitation of SPR in noble metal NPs could cause strong local electric fields that in turn enhance the luminescence emission of QDs. Through altering the distance, the ECL intensities of CdS QDs could be either quenched or enhanced by proximal Au NPs due to the competition between FRET and field enhancement (Shan et al., 2009). The key technique in SPR-ECL study is the spacing distance control on the electrode surface. DNA was usually used since the distance between noble metal and QDs could be exactly calculated through the number of bases. However, the spacing steric of the double stranded DNAs can't be ignored. As a result, the targets conjugated on the double stranded DNAs were crowed and inhibited the binding activity of the targets, leading to the relative poor detection limit (Chen et al., 2014).

In recent years, DNA tetrahedron has been splendid in biological applications because of the three-dimensional scaffold, structural stability, mechanical rigidity, high precision, well-defined spacing and specific orientation (Bergamini et al., 2014; Li et al., 2011; Lu et al.,

* Corresponding author.
E-mail addresses: weizhao@nju.edu.cn (W. Zhao), xujj@nju.edu.cn (J.-J. Xu).

http://dx.doi.org/10.1016/j.bios.2016.11.060
Received 9 August 2016; Received in revised form 14 November 2016; Accepted 25 November 2016
Available online 27 November 2016
0956-5663/

2012; Pei et al., 2014; Yan et al., 2012). DNA tetrahedron possessed the accurate control of recognition units and easily functioned with different chemical moieties or biomolecules, such as metal nanoparticles (Jia et al., 2014; Yan et al., 2012), DNA probes (Li et al., 2015a, 2015b; Miao et al., 2015), fluorescence molecules (Kim et al., 2013a), and antibodies (Yuan et al., 2014). Meanwhile, due to its three-dimensional scaffold, the designed DNA tetrahedron grafting on the electrode surface could reduce the local overcrowding effect with well-defined spacing and enhance spatial positioning range and accessibility of the probes on the electrode surface over linear or stem-loop probe structures (Pei et al., 2010). For example, Zuo's group estimated that the nanospacing between the DNA linker on the top of DNA tetrahedron (5.78 nm edge length) achieved ~5.0 nm (Chen et al., 2014), bringing into 6 times higher capturing amount of horseradish peroxidase than that on the double stranded DNAs based monolayer. In addition, Fan's group further verified that spire DNA tetrahedron-based sensors possessed stronger electrochemistry and fluorescence sensitivity in detection of prostate-specific antigen (Chen et al., 2014), telomerase activity (Li et al., 2015b) and bioactive molecules (Li et al., 2014) than linear DNA or DNA tetrahedron polymer. Recently, Yin's group designed an ECL biosensor with $Ru(phen)_3^{2+}$ as emitter based on tetrahedron structured DNA for the determination of adenosine triphosphate, bringing into low detection limit and sensitivity (Bu et al., 2013).

Inspired by the high mechanical rigidity and structural stability of DNA tetrahedral scaffolds, in this work, a SPR-based ECL sensor was designed using DNA tetrahedral scaffolds as the connector between QDs and metal NPs. CdS QDs-modified electrodes were prepared by dropping and drying CdS QDs suspension on glassy carbon electrode, then thiol-modified DNA tetrahedral scaffolds attached on CdS QDs via Cd-S bond. The telomerase strand primer (TSP) was inserted into DNA tetrahedral scaffolds to perform the telomerase identification with a SPR-based ECL sensor. In the presence of telomerase, TSP would extend at the vertex of DNA tetrahedral scaffolds. Meanwhile, the three-dimensional rigid structure provided higher accessibility and controllability for the assembly of multiple luminol modified Au NPs (L-Au NPs). Once multiple L-Au NPs was introduced to the extended part of TSP on the surface of electrode, the ECL enhancement of CdS QDs was achieved, at the same time a new ECL signal of luminol appeared. The purpose of using two ECL reporters rather than just QDs is to limit the possible interference such as environmental conditions which may influence the signal output and cause false positive or negative error during trace analysis. Compared with single readout system, ratiometric or dual-signal detections have been proved as more accurate methods in biological and environmental analysis (Wu et al., 2016; Zhang et al., 2013). This proposed DNA tetrahedral scaffolds based dual-potential ECL biosensor affords a sensitive and universal platform in bioanalysis.

2. Materials and methods

2.1. Reagents and apparatus

Refer to the Supporting Information.

2.2. Preparation of L-Au NPs and probe DNA-L-Au NPs

Luminol-gold bifunctional nanoparticles (L-Au NPs) with the diameter of 5 nm were prepared according to the previous work (Zhang et al., 2014b). The synthesized L-Au NPs exhibited the characteristic absorption bond of luminol molecule at 250–400 nm and the feature absorption peak at about 525 nm for Au NPs (Fig. S1A, curve c), suggesting the successful synthesis of L-Au NPs.

For the preparation of probe DNA modified L-Au NPs (abbreviated as probe DNA-L-Au NPs), 49 μL probe DNA (1 μM) and 181 μL bbcDNA (10 μM) was pretreated by 10 mM tris(2-carboxyethyl) phosphine hydrochloride (TCEP) to reduce disulfide bonds, then the mixture solution were added to L-Au NPs solution. The obtained solution was stirred for 12 h in order to assure the fastness of Au-S bond. After that, 0.1 M PBS solution containing 3 M NaCl was added to the colloidal solution stepwise and the mixture was finally brought to 0.3 M NaCl. In order to remove excess DNA, the mixture solution was centrifuged and washed with PBS for three times. The final deposition was resuspended in PBS and stored at 4 °C for further use. The assembled probe DNA-L-Au NPs was characterized with UV–vis spectroscopy. When the L-Au NPs were functioned with probe DNA, the UV–vis spectrum showed the characteristic peak of DNA at 260 nm (Fig. S1A, curve d), suggesting the effective assembly of probe DNA-L-Au NPs. Control sequences labelled Au NPs including S7-L-Au NPs, S8-L-Au NPs, S9-L-Au NPs, S10-L-Au NPs, S11-L-Au NPs and S12-L-Au NPs were also assembled using the above procedure.

2.3. Self-assembly of TTSP scaffolds

In this work, DNA tetrahedron inserted with telomerase strand primer (abbreviated as TTSP) scaffolds were formed by one-pot incubation technique with a simple annealing process (Feng et al., 2016; Yan et al., 2012) and the preparation process of TTSP scaffolds was shown in Scheme 1 A. At the beginning, thiol modified three strands (S2, S3, S4) were treated by 10 mM TCEP to cut S-S bond. Then, equimolar amounts of the four constituent oligonucleotides (S1, S2, S3 and S4, the sequences in the Table S1) were combined in TM buffer (10 mM Tris-HCl, 35 mM $MgCl_2$, pH 8.0). The hybridization mixture was heated at 95 °C for 5 min and then cooled to room temperature over 24 h. High temperature could effectively make chain DNA denatured to an open end and improve the hybridization of DNA to expected structures in the process of temperature reduction. Once assembled, the DNA tetrahedron contained TSP at one edge and three thiol groups at other three vertices. Finally, the obtained TTSP scaffolds were purified by ultrafiltration (30 K molecular weight cutoff) to remove the non-conjugated oligonucleotides. In addition, DNA tetrahedral scaffolds (S2S3S4S5, named as T for short) were assembled using the same procedure.

2.4. Gel electrophoresis

Different DNA structures (S1, S2, S3, S4, S1S2S3, S1S2S4, S1S3S4, S2S3S4 and S1S2S3S4) were all incubated at 95 °C for 5 min and then cooled to room temperature over 24 h. Then, the loading samples (the mixture of 7 μL different DNA structures, 1.5 μL 6×loading buffer, and 1.5 μL of UltraPower TM dye) were injected into a 5% native polyacrylamide gel (prepared by 5×TBE buffer). Before injection into the polyacrylamide hydrogel, the loading samples were placed for 3 min. The electrophoresis analysis was run at 100 V for 1 h. After electrophoresis, the obtained board was observed under UV irradiation and photographed with a Molecular Imager Gel Doc XR.

2.5. Preparation of ECL biosensor

The preparation of ECL biosensor was shown in Scheme 1B. Firstly, a glassy carbon electrode (GCE) was applied as a substrate for immobilizing CdS QDs. The synthesis of CdS QDs referred to the Supporting Information. TEM image of the CdS QDs showed an average diameter of 5 nm (Fig. S2), confirming the successful synthesis of CdS QDs. The GCE was polished in sequential order with 1.0, 0.3 and 0.05 μm of alumina power to obtain a mirror-like clean surface and then thoroughly rinsed with distilled water. Subsequently, 10 μL of CdS QDs solution was drop-cast on the surface of GCE and dried to get CdS QDs film modified GCE at room temperature. The obtained CdS QDs-GCE was immersed in TTSP scaffolds solution and incubated overnight at 4 °C to graft the TTSP bioconjugates on the surface of CdS QDs-GCE (named as TTSP-CdS QDs-GCE). After washing with 0.1 M Tris-HCl