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A solid-state electrochemiluminescence biosensing switch for detection of DNA hybridization based on ferrocene-labeled molecular beacon

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ABSTRACT

A solid-state electrochemiluminescence (ECL) biosensing switch incorporating quenching of ECL of ruthenium(II) tris-(bipyridine) ($Ru(bpy)_3^{2+}$) by ferrocene (Fc) has been successfully developed for DNA hybridization detection. The important issue for this biosensing system is based on the ferrocene-labeled molecular beacon (Fc-MB), i.e. using the special Fc-MB to react with the target DNA and then change its structure, resulting in an ECL intensity change. Under the optimal conditions, the difference of ECL intensity before and after the hybridization reaction (ΔI_{ECL}) was linearly related to the concentration of the complementary sequence in the range of 10 fM–10 pM and the detection limit was down to 1.0 fM.

1. Introduction

Sequence-specific DNA detection is extremely important in clinical diagnostics, gene therapy, environmental research, food safety and a variety of biomedical studies [1–5]. A variety of techniques are established for the detection of DNA sequence, such as fluorescence [6–8], chemiluminescence [9–11], electrochemistry [12–15], surface plasmon resonance [16,17], quartz crystal microbalance [18–20] and electrochemiluminescence (ECL) techniques [21–25]. ECL, the generation of an optical signal triggered by an electrochemical reaction, has potential advantages over other methods and has become an important and valuable detection method in biosensors with its versatility, simplified optical setup, very low background signal, good temporal and spatial control [26,27]. Solid-state ECL, viz. immobilizing the ECL substrate on the electrode surface, can reduce the consumption of expensive reagent, enhance the ECL signal, simplify experimental design and create regenerable sensors [28–35]. Therefore, solid-state ECL biosensor is more suitable for biomolecular sensing [36]. However, the fact that the mix of biomolecules with ruthenium(II) tris–(bipyridine) ($Ru(bpy)_3^{2+}$, the most used ECL substrate) would result in a significant loss of the biomolecules' activity, has held the development of solid-state ECL biosensor [36,37]. Thus the development of a highly sensitive solid-state ECL biosensor for the determination of DNA remains a great challenge.

In our recent studies, a solid-state electrochemiluminescence biosensing switch was developed successfully [38–40]. The biosensing system employed $Ru(bpy)_3^{2+}$-AuNPs modified Au electrode to emit ECL, and the ferrocene-labeled molecular beacon (Fc-MB) to control the ECL intensity with consideration of the high ECL quenching efficiency of Fc to $Ru(bpy)_3^{2+}$ [41]. It has been found that the ECL intensity of the $Ru(bpy)_3^{2+}$-AuNPs modified Au electrode was correlated to the conformation of the Fc-MB. Herein, The Fc-MB was designed with special base sequence, which could be combined with its target biomolecule via the reaction of DNA hybridization. During the reactions, double strand DNA (dsDNA) was formed, the Fc-MB opened its stem–loop, and the labeled Fc was consequently kept away from the ECL substrate. Such structural change resulted in an obvious ECL intensity increment due to the decreased quenching effect of Fc to the ECL substrate. The difference of ECL intensity before and after the hybridization reaction (ΔI_{ECL}) can be used to quantitate the complementary sequence rapidly, sensitively and selectively.

2. Experimental sections

2.1. Reagents and apparatus

Oligonucleotides were purchased from Shenggong Bioengineering Ltd Company (Shanghai, China). The sequence of molecular beacon (MB): 5′-NH_2-$(CH_2)_6$-CCCGGTTG*GTGTGGTTGGATTGATCG-TAGGTA*CAACC-$(CH_2)_6$-SH-3′(the underlined and italic sequences

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indicate the stem and loop of the MB); complementary sequence: 3′-CACACCAACCT AACTAGCATCCAT-5′; one-base mismatched sequence: 3′-CACACCAA CCTAACTAGCAT***A***CAT-5′; three-base mismatched sequence: 3′-CAC ***T***CCAACCT***G***ACTAGCAT***A***CAT-5′; noncomplementary sequence: 3′-AGATAAGCATACGACTGAGATT-CA-5′. $Ru(bpy)_3^{2+}$ (99.95%), $HAuCl_4$, 6-mercapto-1-hexanol ($SH-(CH_2)_6-OH$, >97.0%), cysteamine ($SH-(CH_2)_2-NH_2$) and 1-ethyl-3-[(3-dimethylamino)propyl]carbodiimide (EDC) were purchased from Sigma (USA). Ferrocenecarboxylic acid (FCA) was purchased from Maoji Bioengineering Ltd. Company (Shanghai, China). The following buffer solutions were used: 10 mM phosphate buffer solution (PBS) containing 100 mM $LiClO_4$ (pH 7.3), 10 mM PBS containing 0.8 M NaCl and 100 mM $LiClO_4$ (pH 7.3). 20 mM PBS containing 1.0 mM tri-*n*-propylamine (TPrA) and 5.0 mM $LiClO_4$ (pH 8.7) was used as detecting buffer solution. Other reagents were of analytical reagent grade. All of the solutions were prepared with ultrapure water from a Millipore Milli-Q system.

ECL was recorded with MPI-E electrogenerated chemiluminescence analyzer (Xi'an Remax Electronic Science Tech. Co. Ltd.); a CHI 660A electrochemical analyzer (CHI instruments Inc., USA) was used to carry out impedance measurements.

2.2. Preparation of Fc-MB-$Ru(bpy)_3^{2+}$-AuNPs electrode

The Fc-MB-$Ru(bpy)_3^{2+}$-AuNPs electrode was prepared according to previously published protocols [38]. Briefly, a self-assembled monolayer of cysteamine is prepared onto Au electrode firstly (cysteamine-derivated Au electrode), then, the $Ru(bpy)_3^{2+}$-AuNPs composite is assembled onto the cysteamine-derivated Au electrode to form the luminescent substrate. The as-prepared electrode was $Ru(bpy)_3^{2+}$-AuNPs electrode. The Fc-MB is attached onto the above electrode via Au–S interactions, and the electrode was then treated with $SH-(CH_2)_6-OH$ solution to occupy the unassembled surface of $Ru(bpy)_3^{2+}$-AuNPs as well as adjust the Fc-MB distribution on the electrode surface. Thus, the Fc-MB-$Ru(bpy)_3^{2+}$-AuNPs electrode was obtained which has a solid-state electrochemiluminescence biosensing switch of Fc-MB-$Ru(bpy)_3^{2+}$-AuNPs.

2.3. The specific recognition of complementary DNA onto Fc-MB-$Ru(bpy)_3^{2+}$-AuNPs electrode

Hybridization reaction was carried out by incubating the Fc-MB-$Ru(bpy)_3^{2+}$-AuNPs electrode into 500 μL of 10 mM PBS (pH 7.3) containing 0.8 M NaCl, 100 mM $LiClO_4$ and different concentrations of oligonucleotide for 2 h at 20 °C. Then the electrode was washed with the same buffer thoroughly to remove the unhybridized DNA. The dsDNA modified electrode was formed.

2.4. Electrogenerated chemiluminescence detection

A three-electrode system was used with the modified Au electrode (2 mm in diameter) as the working electrode, an Ag/AgCl (sat.) as the reference electrode and a platinum wire as the counter electrode. Cyclic voltammetry mode with continuous potential scanning from 0.0 to 1.2 V and scanning rate of 0.1 V s^{-1} was applied to achieve ECL signal in 20 mM PBS containing 1.0 mM TPrA and 5.0 mM $LiClO_4$ (pH 8.7) at room temperature. A high voltage of −800 V was supplied to the photomultiplier for luminescence intensity determination.

3. Results and discussion

3.1. Recognition of complementary DNA using the solid-state electrochemiluminescence biosensing switch

As displayed in Fig. 1, when Fc-MB is stable in its normal stem–loop structure, the ferrocene molecules approach the surface of the $Ru(bpy)_3^{2+}$-AuNPs electrode, consequently efficiently quenching the ECL signal of $Ru(bpy)_3^{2+}$. While the hybridization occurs between the molecule beacon and the target DNA sequence, the stem of the Fc-MB is opened and then the ferrocene molecules are departed away from the $Ru(bpy)_3^{2+}$-AuNPs electrode, which is expected to result in an increase of the ECL signal due to weakened quenching effect of the ferrocene molecules to $Ru(bpy)_3^{2+}$. Thus, the difference of ECL intensity before and after the hybridization reaction (ΔI_{ECL}) can be used to quantify the complementary DNA.

3.2. The characterization of solid-state electrochemiluminescence biosensing electrode

The fabrication processes of solid-state electrochemiluminescence biosensing electrode and the interaction between fabricated biosensing electrode and complementary DNA were characterized by electrochemical impedance spectroscopy (EIS). For EIS measurements, $[Fe(CN)_6]^{3-/4-}$ was employed as the redox-probe and Nyquist polts were used to calculate the R_{et} for modified electrodes. As shown in Fig. 2, the R_{et} for bare Au electrode was 256.3 Ω, and increased to 329.9, 738.6, and 1102.4 Ω for $Ru(bpy)_3^{2+}$-AuNPs electrode, the Fc-MB-$Ru(bpy)_3^{2+}$-AuNPs electrode and dsDNA electrode, respectively. The corresponding cyclic voltammogram (CV) curves of the electrodes using $[Fe(CN)_6]^{3-/4-}$ as the redox-probe were shown in the inset of Fig. 2. The successive increase in the R_{et} and the CV curves of the electrodes illustrates the successful modification of the biosensing components onto Au electrode. It also indicated that the interaction between the Fc-MB and the complementary ssDNA arose successively.

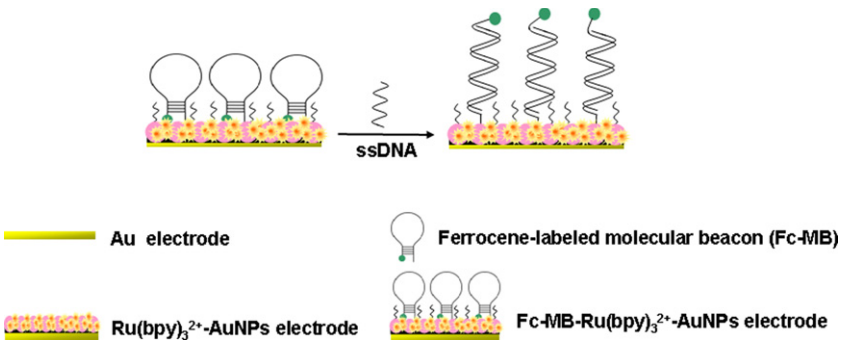

Fig. 1. The scheme of the specific recognition of DNA by using the Fc-MB-$Ru(bpy)_3^{2+}$-AuNPs electrode.