Biosensors and Bioelectronics ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

An electrochemiluminescence biosensor for endonuclease EcoRI detection

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ARTICLE INFO

Article history:
Received 22 November 2015
Received in revised form
11 January 2016
Accepted 28 January 2016

Keywords:
ECL
GNPs-graphene
Amplifier
Endonucleases
EcoRI
$Ru(bpy)_3^{2+}$

ABSTRACT

Endonucleases cleavage of DNA plays an important role in biological and medicinal chemistry. This work was going to develop a reliable and sensitive electrochemiluminescent (ECL) biosensor for detecting endonucleases by using gold nanoparticles graphene composite (GNPs-graphene) as a signal amplifier. Firstly, the GNPs and graphene were simultaneously deposited on the glassy carbon electrode (GCE) by cyclic voltammetry. Then a stem DNA was anchored on the surface of GCE. And with a modifying DNA introduced into the electrode by DNA assembly, a strong ECL signal was obtained. After a DNA modified with ferrocene assembly to the stem DNA, the ECL signal had a sharp decrease due to the quench effect of ferrocene to and the biosensor comes into being a "off" state. With the effect of endonuclease, the ECL signal had a recovery because of the ferrocene being released and the biosensor formed a "on" state. Moreover, the recovery of ECL signal was related to the concentration of endonucleases. Combining specific defined DNA and endonuclease, this method has a potential to detect different endonucleases. In this work, we took the EcoRI as an example to identify the feasibility of ECL biosensor in applying in sensitive detection of endonucleases using a GNPs-graphene signal amplifier. Under optimal condition, the proposed biosensor obtained a low limit of detection (LOD) 5.6×10^{-5} U mL^{-1}. And the stability, selectivity and reproducibility of the biosensor also were researched.

1. Introduction

Endonucleases, a family of nuclease that mainly exists in prokaryotic organisms, are known as "molecular scissors" with highly specific activity in cleaving the phosphodiester bond within DNA at defined positions (Ordinario et al., 2014). The endonucleases have been widely used in PCR assay, gene mapping, medicinal chemistry, enzymatic amplification technique and nanostructures/nanodevices fabrication (Nygen et al., 2005; Nakazato et al., 2006; Kanaras et al., 2007; Langhans and Palladino, 2009). Endonucleases play an important role in prokaryotic organisms with the principal function of protecting host genome against foreign DNA (Galburt and Stoddard, 2002). Therefore, they have been deemed to be important targets in the discoveries of antimicrobial and antiviral drugs (Baughaman et al., 2012; Clercq, 2006; Choi et al., 2003). Accordingly, sensitively and quantitatively assaying endonuclease is critical and useful in drug-development process and disease prevention.

Many works have been done on assaying endonucleases including high-performance liquid chromatography (HPLC), enzyme-linked immunosorben assay (ELISA), gel electrophoresis, fluorescence resonance energy transfer (FRET), fluorescence polarization and gold nanoparticle based colorimetric methods and so on (Alves et al., 1989; Ulubas and Ertunc, 2004; Agarkova et al., 2006; Qian et al., 2014; Huang et al., 2011). Even though those methods are able to detect endonucleases precisely, they exist many disadvantages including sophisticated instrumentations, complicate procedure, high-cost. Recently electrochemiluminescent (ECL) has been well developed in biosensor with both advantages of chemiluminescence and electrochemistry, such as low back-ground signal, being easily controlled and detection (Muzyka, 2014; Yao et al., 2013; Huang et al., 2015). Particularly, the ECL biosensor based on the quenching or enhancement of /TPrA ECL system have been extensively investigated such as the quenching mechanism of /TPrA ECL system by ferrocene and phenol (Miao and Bard, 2003; Wei and Wang, 2011; Xing et al., 2014; Gao et al., 2013a, 2013b). Besides the quenching effect of ferrocene to is better than other (Cao et al., 2006).

The development of ultrasensitive ECL DNA biosensor mainly contributed to the popular strategy of signal amplification. The use

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http://dx.doi.org/10.1016/j.bios.2016.01.082
0956-5663/

of nanomaterial as amplifiers has attracted special interesting in ECL biosensor design such as carbon nanotubes, graphene, gold nanoparticle, nanocomposites etc. (Bertoncello and Forster, 2009; Xu et al., 2011; Wang et al., 2011; Wang et al., 2012a, 2012b). Especially, graphene is a one-atom-thick layer of graphite with two linear bands crossing at the Dirac point. With excellent physical and chemical properties, large surface area and controllable electronic properties graphene has become a promising electrode material in constructing ECL biosensor (Meriga et al., 2015; Ma et al., 2014a, 2014b; Li et al., 2012).

Many methods have been proposed for graphene production, among which the chemical reduction of graphene oxide (GO) obtained from ultrasonic exfoliation of oxidized graphite is the most convenient way to yield large quantities of graphene sheets (Stankovich et al., 2007). However, the practical application of graphene are challenged by its irreversible agglomeration both in the drying state and in common solvents, which significantly reduces its effectiveness. Introducing metal nanoparticles was initially proposed in order to separate graphene sheets. While nowadays, it is well realized that the dispersion of metal nanoparticles on graphene sheets also potentially provides a new way to develop novel catalytic, magnetic, and electronic materials (Xu et al., 2008). Graphene-metal nanocomposite has also been used in ECL biosensor fabrication as a signal amplifier and get a good performance (Wang et al., 2012a, 2012b).

To our best known that few works have been done on assaying endonucleases using an ECL method. In this work, we employed gold nanoparticles-graphene composite (GNPs-graphene) as the ECL biosensor amplifier to detect endonucleases. As shown in Scheme 1, the GNPs and graphene were simultaneously deposited on the glassy carbon electrode (GCE) by cyclic voltammetry (CV). And then a well-designed stem A-DNA modified with thiol was introduced to the surface of electrode by stable Au–S interaction. Followed that a B-DNA modified with $Ru(bpy)_3^{2+}$ and a C-DNA modified with ferrocene were anchored on the surface by DNA self-assembly and the duplex strand, coming into being form A-DNA, B-DNA and C-DNA, would provide a position that the endonucleases can recognize. When the $Ru(bpy)_3^{2+}$ modifying B-DNA joint in the biosensor, a strong ECL signal will be obtained. Because of the quenching effect of ferrocene to $Ru(bpy)_3^{2+}$, the C-DNA modifying ferrocene will cause a signal "off" state. Endonucleases are known as "molecular scissors" with highly specific activity in cleaving the phosphodiester bond within DNA at defined positions. We well defined the A-DNA, B-DNA and C-DNA to make sure that the endonucleases only were cut down at the specific position and released the ferrocene. Under the effect of endonucleases the ferrocene was released and produced a signal "on" state with ECL signal recovery. Therefore the ECL intensity of the DNA biosensor generated a "switch on" mode, which rises with an increase of the concentration of endonucleases, whereby allowing the quantitative detection of endonucleases. This paper takes EcoRI for example to provide a versatile avenue for selective and sensitive detection of different endonucleases by designing different DNA sequence.

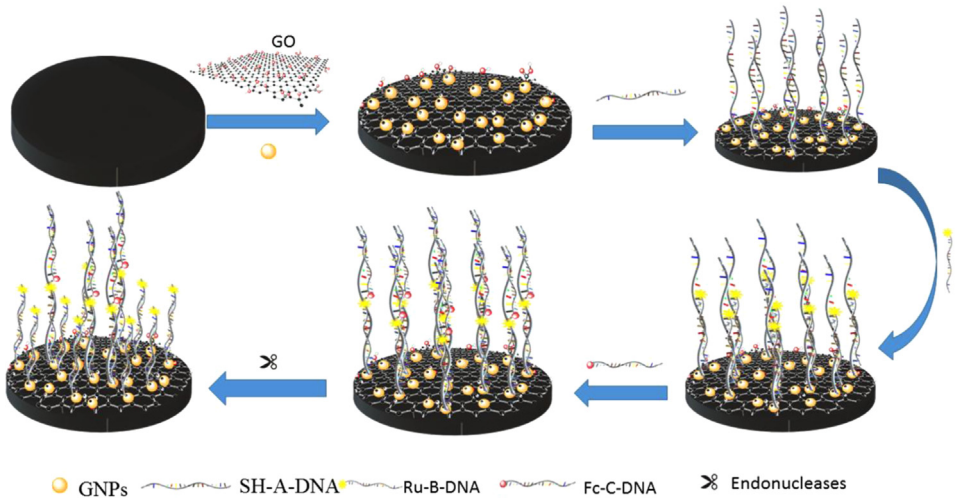
2. Experimental

2.1. Reagents

The DNA oligonucleotide sequences for this experiment are shown below:

A. DNA
5′-SH-$(CH_2)_6$-GGGGTTGGGGAAGGGTACG*A**GG*
AATTCC*GGGTTGGG*-3′
B. DNA: 5′-NH_2-$(CH_2)_6$-CCCTTCCCCAACCCC-3′
C. DNA: 5′-CCCAACCC**GGAATTCC**T-$(CH_2)_6$-NH_2-3′

All oligonucleotides were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The underlining section of the stem A-DNA is complementary to the B-DNA and the italic bases are complementary to the C-DNA. The bold bases are the recognition sequence of EcoRI. The symbol "^" is the position that the endonuclease EcoRI can recognize. Cis-Bis-(2,2′-bipyridine)dichlororuthenium(II) dehydrate (cis-$Ru(bpy)_2Cl_2 \cdot 2H_2O$) were bought from Precious Metal Research Institution (Yunnan, China). 2-mercaptohexanol (MCH) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Tripropylamine (TPrA), N-hydroxysuccinimide ester (NHS), tetrachloroanric (III) acid tetrahydrate [$HAuCl_4 \cdot 4H_2O$], N,N′-dicyclohexyl carbodiimide (DCC), N,N′-dimethlformamide (DMF) were obtained from Aladdin Chemical Reagent Co. Ltd. (Shanghai, China). Endonuclease (EcoRI, EcoRV, PstI, NotI, RsaI, NcoI) and buffer for enzyme reaction were got from Beyotime Biotechnology CO. Ltd. (Shanghai, China). Graphite flakes (325 mesh) was bought from XFNANO material Tech CO. Ltd. (Nan Jing, China). All other chemical not mentioned here were of analytical reagent grade and were used as received. Millipore Milli-Q water (18 MΩ cm) supplied by a Millipore Milli-Q water purification system (Bedford, MA USA) was used throughout. A concentration of 0.1 M phosphate buffer saline (PBS, pH 7.5, 0.1 M NaCl+0.1 M NaH_2PO_4/



Scheme 1. Schematic diagram of the ECL biosensor for detection of endonucleases based on GNPs-graphene amplifier.