Sensors and Actuators B 244 (2017) 885–890

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Electrogenerated chemiluminescence biosensing method for the detection of DNA demethylase activity: Combining MoS_2 nanocomposite with DNA supersandwich

Huiping Sun[a,1], Shangxian Ma[a,1], Yan Li[a,*], Honglan Qi[b,*]

[a] Key Laboratory of Electroanalytical Chemistry, Institute of Analytical Science, Northwest University, Xi'an, Shaanxi 710069, PR China
[b] Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry and Chemical Engineering, Shaanxi Normal University, Xi'an 710062, PR China

ARTICLE INFO

Article history:
Received 5 October 2016
Received in revised form
26 December 2016
Accepted 9 January 2017
Available online 10 January 2017

Keywords:
Electrogenerated chemiluminescence
DNA demethylase
Supersandwich
MoS_2 nanocomposite
Endonuclease

ABSTRACT

We report an ultrasensitive electrogenerated chemiluminescence (ECL) biosening method for the detection of DNA demethylase activity based on the site-specific cleavage of the *Hpa*II endonuclease in conjunction with signal amplification by a Thionin-MoS_2 (Th-MoS_2) nanocomposite and supersandwich DNA. The Thionin (Th) served as an effective mediator for the immobilization of the capture probe onto the surface of MoS_2, after which the long concatamers containing the methylated site 5′-C^{m}CGG-3′ were hybridized with the capture probe, creating a supersandwich DNA structure. Ru(phen)$_3^{2+}$ ECL reagents can be intercalated into the grooves of the formed supersandwich DNA structure with high affinity leading to enhanced signaling. At the biosensing electrode (Ru(phen)$_3^{2+}$/supersandwich DNA/Th-MoS_2/glassy carbon electrode) after demethylation with demethylase, the demethylated supersandwich DNA could be specifically cleaved using *Hpa*II, resulting in a decreased ECL signal from Ru(phen)$_3^{2+}$. An extremely low detection limit of 0.16 pg mL^{-1} was achieved for the detection of DNA demethylase activity. This work demonstrates that the combination of dual signal amplification using a MoS_2-thionin nanocomposite and supersandwich with an inherently sensitive ECL method is a promising tactic for the detection of DNA demethylation and assay of the activity of demethylase.

1. Introduction

DNA methylation is found in the genomes of diverse organisms, including both prokaryotes and eukaryotes [1]. It has a significant physiological role in the epigenetic regulation of gene expression, X-chromosome inactivation, and genomic imprinting [2–4]. The DNA methylation process generally occurs at cytosines in CpG dinucleotides in the mammalian genome and goes along with the catalysis of DNA methyltransferases (MTase) with S-adenosylmethionine (SAM) [5]. On the other hand, DNA demethylation is a reverse process of DNA methylation, in which the methyl group is eliminated from the C-5 position of cytosine in the dinucleotide sequence CpG by DNA demethylase [6]. Over the years, demethylation has been described to play an important role in memory and learning [7], initiating tissue-specific gene expression during embryonic development [8], and removal of the imprinting marks in primordial germ cells [9]. Many methods have been proposed for the detection of DNA demethylase, including fluorescence [10], electrochemical methods [11], and Raman scattering approaches [12]. Although these methods are well-established and effective, they usually pose the challenges of large scale and costly instruments, numerous separation and rinse steps, as well as low sensitivity, which limit their utility for regular assays.

The ECL method has received extensive attention and exhibits distinct merits (such as high sensitivity, low background, and good reproducibility) for the detection of DNA damage, DNA methylation, and cancer cell [13,14]. In spite of these advantages, few studies have reported the utilization of ECL for the use of determining DNA demethylase activity.

Sandwich model has been widely utilized for the identifying of DNA, proteins, cells, and small molecules [15,16]. However, this model was suffered from its low sensitivity because each probe captures only one target molecule. In order to overcome this shortcoming, Plaxco and co-workers reported a novel supersandwich

* Corresponding authors.
E-mail addresses: yanli@nwu.edu.cn (Y. Li), honglanqi@snnu.edu.cn (H. Qi).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.snb.2017.01.065
0925-4005/

type [17]. They made signal probes hybridized with two zones of a target DNA to form a long DNA concatamer, effectively amplifying the signal and achieving higher sensitivity. Comparatively, this modified supersandwich assay could greatly magnify the signal and result in superior sensitivity.

MoS_2 has unique electronic and electrochemical properties: large active area, easy functionalization, and the possibility of surface modification [18]. MoS_2-based nanocomposites usually inherit the advantages of both functionalized nanoparticles and MoS_2, making them attractive for applications in many fields, such as biosensing electrodes, bio-imaging, cancer therapy, and catalysts [19]. It has been previously reported that single-layer MoS_2 can adsorb labeled single-stranded DNA (ssDNA) probes via the van der Waals forces between nucleobases and the basal plane of MoS_2 [20,21]. However, once the ssDNA probe was hybridized with the supplementary target DNA, the nucleobases were buried between the thick negatively charged helical phosphate backbones, due to which the interaction between the double-stranded DNA (dsDNA) and layered MoS_2 was weakened, leading to the separation of dsDNA and the layered MoS_2 [20,21]. The recently reported Thionin (Th) can bind strongly to ssDNA or dsDNA through intercalation and electrostatic interactions [22,23]. Furthermore, it can be attached on the surface of MoS_2 nanosheets to form a Th-MoS_2 nanocomposite for the construction of DNA biosensing electrodes [23,24].

In this study, a novel ECL biosensing method for the detection of DNA demethylase (methyl-CpG-binding domainprotein 2, MBD2) activity was reported based on dual signal amplification by a Th-MoS_2 nanocomposite and supersandwich DNA structure. The assay protocol of the ECL method was depicted schematically in Fig. 1. Capture probe S1 contains a complementary with the Target DNA. Target DNA S2 and complementary sequence S3 contain the methylated fragments of 5′-C/CGG-3′, and can partially hybridize with each other, thus creating long concatamers. Numerous $Ru(phen)_3^{2+}$ can be intercalated into the groove of the double-helix DNA, served as an ECL indicator. Firstly, Th-MoS_2 nanocomposite was modified onto the surface of glassy carbon electrode (GCE). Then, the capture probe S1 was immobilized on the surface of the Th-MoS_2 nanocomposite through intercalation and electrostatic interaction. In the presence of pre-hybridized long concatamers S2&S3, the modified capture probe S1 was hybridized with the S2&S3 leading to the formation of the supersandwich DNA (S1&S2&S3). Next, numerous $Ru(phen)_3^{2+}$ were intercalated into the grooves of supersandwich DNA. In the absence of MBD2, the methylated cytosines were blocked via the methylation of this specific site and could not be specifically cleaved by *Hpa*II endonuclease. In the presence of MBD2, the supersandwich DNA acted as the substrate for MBD2 and could be specifically cleaved by *Hpa*II endonuclease between the two cytosines (recognition site 5′-C|CGG-3′), resulting in a decreased amount of $Ru(phen)_3^{2+}$ on the electrode. Finally, based on the change of the ECL signal of $Ru(phen)_3^{2+}$, the demethylation step was monitored and DNA demethylase activity (MBD2) was studied. The characteristics and analytical performance of the ECL biosensing electrode were presented in this work.

2. Experimental

2.1. Reagents and apparatus

Thionin acetate salt, MoS_2, *N,N*-dimethylformamide (DMF), Tripropylamine (TPrA), Tris(hydroxymethyl) aminomethane (Tris), dichlorotris (1, 10-phenanthroline) ruthenium hydrate ($Ru(phen)_3Cl_2{\cdot}H_2O$), tris(2-carboxyethyl) phosphine hydrochloride (TCEP, 98%) and 1-butyl-3-methylimidazolium hexafluorophosphate were purchased from Sigma-Aldrich. *Hpa*II endonuclease was purchased from New England BioLabs (Ipswich, MA). DNA demethylase MBD2 (methyl-CpG-binding domain-protein 2), MBD1, MBD4, and MeCP2 were purchased from Epigentek Group Inc. (Farmingdale, NY) and stored at −80 °C. The sequences of DNA purchased from Takara (China) were designed according to previously reported literature [25], and their base sequences are as follows: Capture probe (DNA S1), 5'-CGGCACCGGTGGGAGTATTCCGGAGGAAGGTGCCG-3' ; Target sequence (DNA S2), 5'-TACTCCCAC^{m}CGGTGCCGACGGCACCTTCCTCCGGA-3' (the fragment highlighted by dotted lines at the 3′ end is complementary with the dotted line-fragment of the capture probe DNA S1); Complementary sequence (DNA S3), 5'-CGGCACCGGTGGGAGTATTCCGGAGGAAGGTGCCG-3' (the fragments shown by wavy lines and dotted lines are complementary with the correspondingly highlighted fragments of DNA S2); Single-base mismatched sequence (DNA S4): 5′-TACTCCCAC^{m}CGGTGCCGACGGCACCTTCCTC***T***GGA-3′ (The mismatched base ***T*** is italicized and bolded). Four oligonucleotides were dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and refrigerated at −20 °C.

The experimental set-up for ECL measurements was the same as that reported in the previous paper [26] The ECL emission was detected by using a model MPI-A electrochemiluminescence analyzer that is produced by Xi'an Remex Electronics (Xi'an, China), and the voltage of the PMT was set at −600 V by pulsing the applied potential from 0.2 to 1.50 V (vs. Ag/AgCl) with a scan rate of 50 mV s^{-1} throughout the process. The conventional three-electrode cell was used, which included a platinum wire as a counter electrode, an Ag/AgCl (3.0 M KCl) as reference electrode and a modified glassy carbon electrode or bare glassy carbon electrode as working electrode.

2.2. Fabrication of biosensing electrode

First, a glassy carbon electrode (GCE, 3.0 mm diameter) was polished with 0.3 and 0.05 μm-alumina slurry (Beuhler) successively, followed by thorough rinsing with water. Then, the Th-MoS_2 nanocomposite was prepared according to reported procedures with Th binding to the S edge of the MoS_2 nanosheets and added to DMF with a final concentration of 2 mg mL^{-1} [23]. Next, 20 μL of this solution was spin-coated with a microsyringe onto the surface of the cleaned GCE, and dry naturally at room temperature to obtain the Th-MoS_2 modified GCE (Th-MoS_2/GCE) [23]. This modified electrode was then washed with deionized water to avoid the possible influence of the residual DMF on the following experiments. Next, the Th-MoS_2/GCE was modified with S1 by immersion in 10 μL of 1 μM S1 (in a fixed buffer consisting of 10 mM Tris, 1.0 mM EDTA, 1.0 M NaCl, and 1.0 mM TCEP, with pH 7.0) for 2 h at the room temperature to obtain S1/Th-MoS_2/GCE [24]. The electrode was then washed with deionized water, dried with N_2, and incubated with 10 μL of partially hybridized S2&S3 (1 μM in 10 mM Tris, 1.0 mM EDTA, 1.0 M NaCl, pH 7.4) for 2 h at 37 °C to form S2&S3/S1/Th-MoS_2/GCE. For the preparation of the ECL biosensing electrode, a droplet of 10 μL of 2 mM $Ru(phen)_3^{2+}$ was placed onto the S2&S3/S1/MoS_2-thionin/GCE for 5 h at 37 °C and then washed thoroughly with TE buffer and water.

2.3. ECL measurements

Demethylation of the S2&S3/S1 superhybrid was performed according to previous reports at 37 °C for 2 h at the 5′-C^{m}CGG-3′ site by incubating the ECL biosensing electrode with 5 μL of 50 mM Tris-HCl buffer (pH 8.0) containing different activities (concentrations) of MBD2 (from 0.5 to 500 pg mL^{-1}) [11]. Subsequently, the demethylated S2&S3/S1superhybrid modified electrode was