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Short communication

Electrogenerated chemiluminescence biosensing method for the detection of DNA methylation and assay of the methyltransferase activity

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ABSTRACT

A novel electrogenerated chemiluminescence (ECL) biosensing method for highly sensitive detection of DNA methylation and assay of the CpG methyltransferase (M. *Sss*I) activity was developed on basis of enzyme-linkage reactions and ruthenium complex served as an ECL tag. The ECL biosensing electrode was fabricated by self-assembling 5′-thiol modified 32-mer single-strand DNA (ss-DNA)-tagged with ruthenium bis (2,2′-bipyridine) (2,2′-bipyridine-4,4′-dicarboxylic acid)-ethylenediamine on the surface of a gold electrode, and then hybridized with complementary ss-DNA to form duplex DNA (ds-DNA). When M. *Sss*I and S-adenosylmethionine were introduced, all cytosine residues within 5′–CG-3′ of ds-DNA on the biosensing electrode were methylated. After the methylated biosensing electrode was treated by *Hpa*II endonuclease, the un-methylated cytosines were cleaved, thus led to decrease ECL signal. The ECL intensity of ECL biosensing electrode is related to the methylation level and M. *Sss*I activity in a fixed concentration *Hpa*II endonuclease. The increased ECL intensity was direct proportion to M. *Sss*I activity in the range from 0.05 to 100 U/mL with a detection limit of 0.02 U/mL. This work demonstrates that the combination of the enzyme-linkage reactions with a highly sensitive ECL technique is a great promising approach for the detection of DNA methylation level, assay of the activity of MTase, and evaluation of the capability of inhibitors for the methyltransferase.

1. Introduction

DNA methylation (Diede et al., 2010), a critical process existing in both prokaryotes and eukaryotes, is achieved by the catalysis of DNA methyltransferases (MTase) in the presence of S-adenosylmethionine (SAM). Methylation of the position 5 of cytosine in DNA is a primary epigenetic mechanism and plays crucial roles in gene transcription, embryogenesis, and diseases (Weinhold, 2006). Recent studies have demonstrated that aberrant methylation of CpG islands in promoter regions of genes is a new generation of cancer biomarkers, and can be regarded as a hallmark of diseases and particular cancer (Moshe, 2005). Moreover, DNA MTase is a novel family of pharmacological targets for the treatment of tumors (Esteller et al., 2001; Esteller, 2002). Great efforts have been made to improve the sensitivity of DNA methylation and assay of MTase activity. In particular, the detection of site-specific CpG methylation undoubtedly can be used for determining specific cancer types, providing insights into the mechanism of gene repression and discovering new drugs to treat methyl-CpG-related cancer (Poeter et al., 2007; Choy et al., 2010). Some methods for the detection of DNA methylation and assay of MTases activity have been established including radioactive labeling of [methyl-^{3}H]–SAM, polymerase chain reaction (PCR) (Lyko et al., 2000; Hatada et al., 1991), fluorescence (Feng et al., 2008; Wang et al., 2009; Li et al., 2007), light scattering (Zhao et al., 2010), colorimetric (Liu et al., 2010; Li et al., 2010; Song et al., 2009), capillary electrophoresis (Fraga et al., 2000), high-performance liquid chromatography (HPLC) (Friso et al., 2002), and electrochemical methods (Wang et al., 2010; Liu et al., 2011). The above methods usually challenged with some shortcomings, such as time-consuming, DNA-consuming, laborious treatment and lower sensitivity. Thus the development of a highly sensitive and simple method for DNA methylation detection and MTases activity assays is greatly required.

Electrogenerated chemiluminescence (ECL) method has many advantages such as simplicity, high sensitivity, rapidity and easy controllability, and has been proved to be very useful in immunoassay, DNA hybridization assays and enzymatic biosensors (Miao, 2008; Zhang et al., 2008; Hu and Xu, 2010; Li et al., 2011). However, ECL biosensing methods for the detection of DNA methylation and assay of the methyltransferase activity have not been reported.

The aim of present work is to develop a highly sensitive ECL biosensing method for the detection of DNA methylation and

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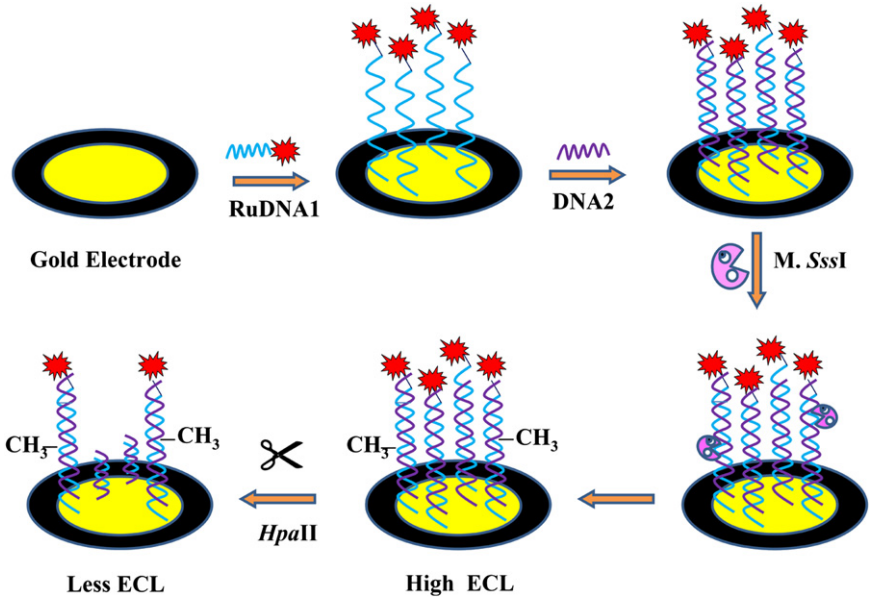

Fig. 1. Schematic diagram of the ECL biosensing method for the detection of DNA methylation and assay of MTase activity.

assay of the CpG MTase (M. *Sss*I). A schematic diagram of the ECL biosensing principle for the detection of DNA methylation and assay of MTase activity is shown in Fig. 1. In this paper, the fabrication of the ECL biosensing electrode and the analytical performance for the detection of DNA methylation and assay of the CpG MTase (M. *Sss*I) were presented.

2. Experimental

2.1. Reagents and apparatus

Ruthenium bis(2,2′-bipyridine) (2,2′-bipyridine-4,4′-dicarboxylic acid)-*n*-hydroxysuccinimide ester [Ru(bpy)$_2$(dcbpy)–NHS], tris (hydroxymethyl)aminomethane (Tris), tris(2-carboxyethyl) phosphine hydrochloride (TCEP, 98%), dithiothreitol (DTT), bovine serum albumin (BSA), 5-azacytidine (5-Aza), and 5-aza-2′-deoxycytidine (5-Aza-dC) were purchased from Sigma-Aldrich and used as received. S-adenosylmethionine (SAM), *E.coli* CpG methyltransferase (M. *Sss*I), and *E.coli* restriction endonuclease (*Hpa*II) were obtained from New England BioLabs (Ipswich, MA). Millipore Milli-Q water (18 ΩM cm) was used throughout. The synthetic oligonucleotides were purchased from Shenggong Bioengineering Co. Ltd. (Shanghai, China), and their base sequences are as follows: 5′–SH-(CH$_2$)$_6$-CCT CTG TGC G**CC GG**T CTC TCC CAG GAC AGG CA-(CH$_2$)$_6$-NH$_2$-3′ (probe DNA, DNA1); 5′–TG CCT GTC CTG GGA GAG A**CC GG**C GCA CAG AGG-3′ (Complementary DNA, DNA2); 5′–TG CCT GTC CTG GGA GAG AC**T** GGC GCA CAG AGG-3′ (One-base mismatched DNA, DNA3).

The experimental set-up for ECL measurement was same as the previous paper (Zhang et al., 2008) (see more details in the Supplementary data).

2.2. Fabrication of biosensing electrode

The ECL probe, 5′-thiol modified 32-mer DNA (DNA1)-tagged with Ru(bpy)$_2$(dcbpy)–NHS (abbreviated as RuDNA1), was synthesized according to literatures (Zhang et al., 2008) with some modifications (see more details in the Supplementary data). A gold disk electrode (ϕ=2.0 mm) was cleaned according to the reported protocol (Zhang et al., 2008). The cleaned electrode was thoroughly rinsed with water and then immersed in 500 μL of 6.5×10^{-6} M RuDNA1 for 4 h at 37 °C. After that the modified electrode was washed twice with water to remove the physically adsorbed ECL probe. The RuDNA1 modified electrode was immersed in 500 μL of 3.3×10^{-6} M DNA2 for 1 h to form the sensing interface, and then was washed with water. This hyzdized electrode was used as an ECL biosensing electrode.

2.3. ECL measurements

The M. *Sss*I assay solution was prepared by mixing Tris–HCl buffer with SAM solution and M. *Sss*I solution to a final concentration 10 mM Tris–HCl, 10 mM DTT, 10 mM $MgCl_2$, 50 mM NaCl, 160 μM SAM and different activity (concentration) of M. *Sss*I (pH 7.9). For the detection of M. *Sss*I, an ECL biosensing electrodes fabricated was immersed in 500 μL of the solution prepared above and incubated at 37 °C for 2 h to form the methylation RuDNA1/DNA2 hybrid. Subsequently, the methylation RuDNA1/DNA2 hybrid modified electrode was immersed in 500 μL of 10 mM Tris–HCl buffer (pH 7.40) containing 20 U/mL *Hpa*II, 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 200 μg/mL BSA, and 50% glycerol for 2 h at 37 °C to cleave the un-methylated cytosines, washed with the 10 mM Tris–HCl buffer (pH 7.40). Finally, the cleaved electrode was transferred into 2.0 mL of 0.10 M PBS (pH 7.40) containing 0.02 M TPA to record the ECL response. A linear potential with a scan rate of 100 mV/s was applied from 0 to +1.25 V (vs. Ag/AgCl) and ECL signal was recorded. The activity of M. *Sss*I was quantified by the increased ECL intensity.

For evaluation of the inhibition effects of two representative anticancer drugs (5-Aza and 5-Aza-dC) on the M. *Sss*I activity, the hybridized electrode prepared above was immersed in 500 μL of MTase assay solution containing 500 U/mL M. *Sss*I and different concentration of the inhibitors at 37 °C. The ECL measurement was the same as for the detection of M. *Sss*I.

3. Results and discussion

3.1. Characterization of the ECL biosensing electrodes

The electrodes in each step of the fabrication of the ECL biosensing electrode were characterized by electrochemical