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One-pot synthesis of GO/AgNPs/luminol composites with electrochemiluminescence activity for sensitive detection of DNA methyltransferase activity

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ABSTRACT

DNA methyltransferases catalyze the transfer of a methyl group from S-adenosylmethionine to the target adenine or cytosine, eventually inducing the DNA methylation in both prokaryotes and eukaryotes. Herein, we developed a novel electrochemiluminescence biosensor to quantify DNA adenine methylation (Dam) methyltransferase (MTase) employing signal amplification of GO/AgNPs/luminol composites to enhance the assay sensitivity. The method was developed by designing a capture probe DNA, which was immobilized on gold electrode surface, to hybridize with azide complementary DNA to form the azide-terminated dsDNA. Then, alkynyl functionalized GO/AgNPs/luminol composites as the signal probe were immobilized to azide-terminated dsDNA modified electrode via click chemistry, resulting in a high electrochemiluminescence (ECL) signal. Once the DNA hybrid was methylated (under catalysis of Dam MTase) and further cleaved by Dpn I endonuclease (a site-specific endonuclease recognizing the duplex symmetrical sequence of 5′-G-Am-T-C-3′), GO/AgNPs/luminol composites release from the electrode surface to the solution, leading to significant reduction of the ECL signal. The change of the ECL intensity is related to the methylation status and MTase activity, which forms the basis of MTase activity assay and site-specific methylation determination. This novel strategy can be further used as a universal method for other transferase determination by designing various transferase-specific DNA sequences. In addition, this method can be used for the screening of antimicrobial drugs and has a great potential to be further applied in early clinical diagnosis.

1. Introduction

DNA methylation, which refers to methyltransferases (MTase)-catalyzed covalent addition of a methyl group to adenine or cytosine residues in the specific DNA sequence (Lee et al., 2010), is a critical process existing in both prokaryotes and eukaryotes. DNA methylation occurs at the C-5/N-4 positions of cytosine and at the N-6 position of adenine and is catalyzed by DNA MTases (Okano et al., 1999; Palmer and Marinus, 1994). A number of human diseases have been found to be associated with aberrant gene methylation (Baylin et al., 2001; Costello et al., 2000). During this aberrant methylation process, the DNA MTase acts as a crucial participator to transfer a methyl group from S-adenosylmethionine (SAM) to the N-6 position of adenine in 5′-G-A-T-C-3′ (Schmitt et al., 1997). Recent studies have testified that aberrant DNA methylation influences the interaction between DNA and protein, and alters gene expression, which may result in tumor occurrence and tumor growth (Shames et al., 2007). Notably, the DNA MTases have been treated as a potential target for anticancer drugs (Heithoff et al., 1999; Low et al., 2001). Therefore, the development of a sensitive method for the MTase assay is of significance for both fundamental biochemical research and drug discovery.

In these years, compared to high-performance liquid chromatography (Reenilä et al., 1995), radioactive labeling (Som and Friedman, 1991), fluorescence (Feng et al., 2007; Li et al., 2007a), and colorimetric analysis systems (Li et al., 2010; Zheng et al., 2013), DNA methyltransferases activity assays based on electrochemical methods have been developed quickly because of the advantages of cheap instruments, simple operation, easy separation, and high sensitivity and selectivity. Electrochemical biosensors have been designed lately for the detection of DNA methyltransferases activity by measuring the current and charge responses of redox probes conjugated during the DNA methylation processes (He et al., 2011a; Li et al., 2012a; Liu et al., 2011). Liu

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et al. reported the quantitative measurements of methyltransferase activity based on oxidation current of ferrocene (Liu et al., 2011). To amplify the electrochemical response and improve the sensitivity, thionine/GO composites labeled dsDNA for the methyltransferases assay was also designed by measuring the redox currents of thionine, yet a low sensitivity was achieved (Li et al., 2012a). A sensitive and simple signal-on electrochemical assay for detection of Dam methyltransferase (Dam MTase) activity based on DNA-functionalized gold nanoparticles (AuNPs) amplification coupled with enzyme-linkage reactions was presented (He et al., 2011a). Despite the improvement of these methods, sophisticated procedures of electroactive labeling are needed, and sometimes, the performances such as linear range and sensitivity were also limited. Therefore, it is still a challenge in developing sensitive, rapid, accurate, and simple methods for the profiling of Dam MTase activity and inhibition.

Electrogenerated chemiluminescence (ECL) is a light emission process in a redox reaction of electrogenerated reactants, which combines the electrochemical and luminescent techniques (Richter, 2004). Compared to the conventional electrochemical methods and luminescence techniques, the ECL technique not only shows high sensitivity and wide dynamic concentration response range but also is potential and spatial controlled. ECL biosensor is a powerful device for ultrasensitive biomolecule detection and quantification by combining the selectivity of the biological recognition elements and the sensitivity of ECL technique and is widely used in immunoassay (Jie et al., 2008; Liu and Ju, 2008), DNA analysis (Duan et al., 2010; Hu et al., 2009), environmental detection and clinic diagnostics (Miao and Bard, 2004; Wang et al., 2009). In spite of its excellent properties, few studies have been conducted concerning the detection of DNA methyltransferase activity and inhibition by ECL methods (Li et al., 2012b, 2013).

Functional nanomaterials have attracted considerable interest motivated by their promising applications ranging from chemistry to life sciences, materials, nanosciences, engineering sciences, and environmental sciences (Daniel and Astruc, 2003; Klajn et al., 2010). Gold nanomaterials possess excellent stability, good biocompatibility, ease of self-assembly and unique optical, catalytic, redox-reactive, electrochemical, and surface properties (Daniel and Astruc, 2003). To date, a series of functionalized gold nanomaterials with novel magnetic, surface-enhance Raman scattering and catalytic properties have been successfully synthesized (Debouttière et al., 2006; Kisailus et al., 2005; Qian et al., 2009). However, few studies have been reported regarding nanomaterials with novel chemiluminescent (CL) property. Recently, Chai et al. synthetized luminol functionalized AuNPs as ECL tags for ultrasensitive DNA assay (Chai et al., 2010). He et al. employed luminol–AgNPs labeling to detect M. tuberculosis DNA, and the as-prepared luminol–AgNPs exhibited much better CL/ECL activity than previously reported luminol–AuNPs (He et al., 2011b). In addition, graphene oxide (GO), a two-dimensional derivative of graphene, which consists of an atomically thin sheet of graphite, has recently attracted much attention due to its unique optical, thermal, mechanical, and electrochemical properties (Chen et al., 2012b; Dreyer et al., 2010). To further expand their application fields, the preparation of nanocomposites based GO with unique properties is very significant. For example, Cui and coworkers synthesized lucigenin functionalized Pt nanoparticles/reduced graphene oxide nanocomposites with chemiluminescence activity (He and Cui, 2012a). Then, He et al. synthesized luminol functionalized sliver nanoparticles/graphene oxide composites for “signal on” chemiluminescent detection of glutathione activity, with good sensitivity and low detection limit (He and Cui, 2012b). In spite of their excellent properties, few studies have been conducted concerning the detection of MTase activity and inhibition base on luminol functionalized sliver nanoparticles/graphene oxide composites as ECL labeling.

In the present work, the proof-of-concept of a novel ECL biosensor for the Dam MTase activity and inhibition analysis using highly chemiluminescent GO/AgNPs/luminol composites to assemble large amount of luminol for amplifying ECL signal was demonstrated. Firstly, azide-terminated complementary DNA was hybridized on the capture probe DNA modified gold electrode surface to form azide-terminated duplex DNA modified electrode. The alkynyl functionalized GO/AgNPs/luminol composites were then assembled on the modified electrode surface via click chemistry for ECL signal generation and amplification. Once the DNA adenine methylation methyltransferase and methylation-responsive restriction endonuclease Dpn I are presented, the ECL signal would be significantly reduced by releasing GO/AgNPs/luminol from the electrode surface into solution due to the digestion of dsDNA at the site of 5′-G-Am-T-C-3′. The ECL signal change is related to the methylation status and MTase activity, which forms the basis of MTase activity assay and site-specific methylation determination.

2. Materials and methods

2.1. Materials, apparatus and synthesis of graphene oxide sheets

Materials, apparatus and synthesis of graphene oxide sheets were presented in Supplementary material.

2.2. Synthesis of alkynyl functionalized GO/AgNPs/luminol composites

In a typical synthesis (He and Cui, 2012b), ethanol/H_2O solution containing 9 mL absolute ethanol and 5 mL ultrapure water, 2 mL of 5 mmol/L $AgNO_3$ and 0.33 mL of 1 mg/mL GO aqueous solution were placed in a 50 mL beaker flask fitted with a magnetic stirrer, and then 0.5 mL of 0.01 mol/L luminol was quickly added to the mixed solution under stirring and was reacted for 4 h at room temperature. During this time, the color of the reaction solution was gradually changed from light yellow to deep yellow, indicated that the GO/AgNPs/luminol composites were successfully synthesized. In this strategy, luminol was not only used as reducing agent for the effective reduction of $AgNO_3$ to AgNPs, the excess luminol was also be used as stabilizers directly coated onto the AgNPs surface through the Ag–N covalent interaction (He et al., 2011b) and in addition used as ECL indicator immobilized on the surface of GO by π-π stacking and hydrogen bonding (He and Cui, 2012b). The obtained GO/AgNPs/luminol composites were centrifuged (12 500 rpm) twice and redispersed with 0.1 mol/L NaOH solution. 1 mM propargylamine was added to 0.2 mL GO/AgNPs/luminol composites and incubated at room temperature for 3 h to form the alkynyl functionalized GO/AgNPs/luminol composites. Then, the obtained composites were centrifuged at 12500 rpm for 10 min and the precipitates were redispersed with 200 μL click chemistry solution containing $CuSO_4 \cdot 5H_2O$ (1.0 μM), TBTA (1.1 μM) and sodium L-ascorbate (4.0 μM) in H_2O/MeOH/THF (2:2:1 v/v/v) (Krishna and Caruthers, 2012).

2.3. Modification of gold electrode with ECL reagent via click chemistry

A gold electrode (diameter of 2 mm) was polished carefully with 1.0, 0.3, and 0.05 μm α-Al_2O_3 powder on fine abrasive paper and then thoroughly rinsed with ethanol and water. Prior to immobilizing DNA, the gold electrode was scanned in 0.5 M H_2SO_4 between −0.2 and 1.55 V (vs Ag/AgCl) at 100 mV/s until a