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A label-free electrochemical biosensor for methyltransferase activity detection and inhibitor screening based on graphene quantum dot and enzyme-catalyzed reaction

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ABSTRACT

A label-free electrochemical biosensor for methyltransferase (M.SssI MTase) activity detection and inhibitor screening was developed based on the amplification of graphene quantum dot (GQD) and enzyme-catalyzed reaction. In the construction of the electrochemical biosensor, auxiliary DNA hybridized with capture DNA to form the double-stranded DNA structure which contained a specific recognition sequence (5′-CCGG-3′) for both M.SssI MTase and restriction endonuclease HpaII. The presence of M.SssI MTase caused the methylation of CpG site in the ds-DNA, which could not be digested by HpaII, causing GQD bound to the undigested ds-DNA and acted as new platform for the immobilization of horseradish peroxidase (HRP) through noncovalant assembly. The modified HRP catalyzed the hydrogen peroxide-mediated oxidation of 3, 3′, 5, 5′-tetramethylbenzidine, resulting an electrochemical signal output. The proposed biosensor realized sensitivity detection of M.SssI MTase activity in the range of 1–40 U mL^{-1} with a detection limit of 0.3 U mL^{-1}, and this dual-amplified biosensor had been successfully applied in M.SssI MTase activity inhibitor screening of procaine and epicatechin. Furthermore, this proposed strategy can also be extended for other methyltransferase detection, which had a promising application in clinical diagnosis and drug development.

1. Introduction

Deoxyribonucleic acid (DNA) methylation is one of the most prominent forms of epigenetic regulation and plays crucial roles in the regulation of gene transcription, gene expression, genomic imprinting, genomic stability, cellular differentiation, and cell development [1–5]. The DNA methylation is catalyzed by specific DNA methyltransferases (M.SssI MTase) which can transfer a methyl group from the donor S-Adenosylmethionine (SAM) to cytosine residues in the 5′-position of CpG dinucleotides (5′-CG-3′) [6–8]. Under usual circumstances, aberrant DNA methylation is always related to cancer because it keeps the tumor suppressor genes silent [9], which resulted in the change of normal cellular functions and phenotypes [10]. Moreover, the alterations of DNA methylation are linked to the abnormal expression/activity of DNA MTase [11,12], thus DNA MTase may become a potential target for anticancer therapy and drug screening [13,14]. Especially, the alterations in M.SssI MTase activity usually occur far before other signs of malignancy and may be of potential use in early diagnosis of tumors [3]. It is reported that aberrant DNA methylation is recognized as a potential biomarker in early diagnosis of cancer, and also regarded as hallmark of other diseases [15,16]. Therefore, it is of great significance to develop simple and sensitive strategies for MTase activity assays.

Commonly-used methods for DNA MTase activity detection include radioactive labeling strategy [17], high performance liquid chromatography [18], gel electrophoresis [19], and immune-based assay [20,21]. However, these methods are involved in radio-labeled substrates, expensive equipments, laborious and time-consuming operation or the use of antibodies. Compared to these methods, electrochemical methods have attracted great attention because of their low cost, simplicity, rapidity and high sensitivity. Zhao and his group developed a methylation-specific electrochemical biosensing strategy for highly sensitive and quantitative analysis of promoter methylation [22], while the method was lack of sensitivity. For different kinds of biosensors, carbon nanomaterials were used to further amplify electrochemical signals and enhance sensitivity.

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Graphene quantum dot (GQD), as recently emerging carbon-based materials, which is quasi-zero-dimensional graphitic nanocrystal less than 20 nm [23]. GQD has attracted increasing attentions due to its special advantages, such as low toxicity, high chemical stability, robust chemical inertness, excellent water solubility and suitability [24–28]. Due to its edge properties, the carboxylic acid moieties at the edge of GQD are easy to be functionalized with various groups [29]. Based on the formation of an amide bond between a carboxyl group and an amino group, the GQD can easily modified on the electrode by interacting GQD with amino-terminated DNA. In addition, due to the large surface-to-volume ratio, excellent compatibility of GQD, large amount of enzyme could immobilized on GQD through noncovalent assembly to produce signal [30,31]. Therefore, GQD has great potential of application in the construction of electrochemical biosensor.

Most extensively research methods are based on methylation-sensitive restriction endonuclease such as HpaII-MspI (5′-CCGG-3′), SmaI-XmaI (5′-CCCGGG-3′), DpnI-Dam (5′-GATC-3′), and so on [32–34]. Among them, restriction endonuclease HpaII could recognize the 5′-CCGG-3′ sequence in double-stranded DNA (ds-DNA) and then catalyzed the cleavage process. The CpG sites are regions of DNA where a cytosine nucleotide followed by a guanine nucleotide in the linear sequence of bases along its 5′ to 3′ direction. CpG is shorthand for 5′-C-phosphate-G-3′, that is, cytosine and guanine are separated by only one phosphate; phosphate links any two nucleosides together in DNA. The methylation of the specific CpG dinucleotides will block the CpG dinucleotide site in the 5′-CCGG-3′ sequence, higher M.SssI MTase activity leads to more CpG sites being methylated and impedes the restriction endonuclease HpaII digestion process. Therefore, the restriction endonuclease HpaII has been frequently used in M.SssI MTase activity assays.

In this work, a label-free and dual-amplified electrochemical biosensor for sensitive and selective detection of DNA MTase activity was developed based on GQD and HRP-catalyzed reaction. The capture DNA hybridized with auxiliary DNA to form the ds-DNA structure which contained a specific recognition sequence (5′-CCGG-3′) for both M.SssI MTase and HpaII. The presence of M.SssI MTase methylated the CpG site of ds-DNA and impeded the digestion process of HpaII. Then GQD interacted with the undigested ds-DNA and acted as a new platform for immobilization of horseradish peroxidase (HRP), HRP-catalyzed reaction resulted in electrochemical signal output. This dual-amplified biosensor exhibited good sensitivity and selectivity towards M.SssI MTase activity detection, which could be an effective tool in clinical diagnosis.

2. Experimental sections

2.1. Reagents and materials

CpG methyltransferase (M.SssI MTase), S-Adenosylmethionine (SAM), restriction endonucleases HpaII, HaeIII and AluI were purchased from New England Biolabs Inc. (Beijing, China). GQD was purchased from XFNANO Materials Tech Co., Ltd. (Nanjing, China). HRP was purchased from Aladdin Industrial Corporation 16050 (USA). N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), 30% H_2O_2 and 3, 3′, 5, 5′-tetramethylbenzidine (TMB) were purchased from Aladdin Reagent Company (Shanghai, China). 6-mercapto-1-hexanol (MCH) were purchased from Sigma-Aldrich (USA). All other reagents were analytical reagent. Phosphate buffered saline (PBS, pH 7.4) was used as supporting electrolyte, and 20 mM Tris-HCl (pH 7.4) containing 140 mM NaCl, 5 mM $MgCl_2$ was used to prepare an aptamer solution. All aqueous solutions were prepared with ultrapure water (18.25 MΩ cm) produced by an Aquapro water purification system.

Oligonucleotides were obtained from Sangon Biotech Co., Ltd. (Shanghai, China). The sequences of these oligonucleotides were as follows:

Capture DNA: 5′-NH_2-$(CH_2)_6$-TTT TTC AGT CCG GAG GTG AAC CTT-$(CH_2)_6$-NH_2-3′

Auxiliary DNA: 5′-ACC TCC GGA CTG-3′

2.2. Apparatus

All electrochemical measurements including cyclic voltammetry (CV) and electrochemical impedance spectroscopic (EIS) were carried out with a CHI 660E electrochemical workstation (Chenhua, Shanghai) using a conventional three-electrode system that consisted of a platinum wire electrode as auxiliary electrode, a saturated calomel electrode (SCE) as reference electrode and a 2-mm diameter gold electrode (GE) as working electrode. Transmission electron microscopy (TEM) image were obtained through Tecnai G20 transmission electron microscope (FEI company, Hillsboro, USA). Amperometric detection was performed in PBS (pH 7.4) solution containing 1.0 mM H_2O_2 and 15 μL of 100 mM TMB at the potential of 150 mV.

2.3. Preparation of electrochemical biosensor

The bare GE was polished carefully with 0.05 μm alumina powder, followed by sequential sonication in distilled water and ethanol for 5 min. Before modification, GE was scanned in 0.5 M H_2SO_4 from − 0.2 to 1.6 V by CV at a scan rate of 100 mV s^{-1}, until a stable voltammetric peaks was obtained. After rinsed thoroughly with ultrapure water and dried with nitrogen, the cleaned GE was immediately incubated with 1 μM capture DNA for 12 h at room temperature. Then capture DNA modified GE was immersed in 1 mM MCH for 1 h to obtain a well-aligned DNA monolayer. Then the modified electrode was incubated with 1 μM auxiliary DNA to form the double-stranded DNA (ds-DNA) containing a specific recognition sequence (5′-CCGG-3′) for both M.SssI MTase and HpaII. Whereafter, the electrode was incubated with 50 μL mixture solution containing 10 μL SAM and different concentration of M.SssI MTase, and then the electrode was treated with 60 U mL^{-1} HpaII. After the completion of cleavage process, the electrode was immersed in 2 mg mL^{-1} GQD solution containing EDC (400 mM) and NHS (100 mM) for 1 h. After the modified electrode was immersed into 2 mg mL^{-1} HRP solution, the amperometric detection was performed in 10 mM PBS (pH 7.4) containing 1.0 mM H_2O_2 and 15 μL of 100 mM TMB.

3. Results and discussion

3.1. Principle of the electrochemical biosensor

The label-free electrochemical biosensor for methyltransferase activity detection was based on the amplification of GQD and enzyme-catalyzed reaction. As shown in Fig. 1, capture DNA self-assembled on the surface of GE through Au-N binding [35]. MCH was used to block the electrode and prevent nonspecific adsorption. Then capture DNA hybridized with auxiliary DNA to form the double-stranded DNA (ds-DNA) which contained a specific recognition sequence (5′-CCGG-3′) for both M.SssI MTase and endonuclease HpaII. Endonuclease HpaII could recognize the specific sequence and catalyzed the cleavage of ds-DNA. M.SssI MTase could catalyze the methylation process of ds-DNA containing the specific CpG dinucleotides, which block the CpG dinucleotide site of ds-DNA. Because of the methylated ds-DNA could not be digested by HpaII, higher M.SssI MTase activity led to more ds-DNA remained on GE, and more GQD could interact with capture DNA (The characterization of GQDs is shown in Fig. S1, indicating the size of GQD was about 3 nm in diameter). Due to the large surface-to-volume ratio and excellent compatibility of GQD, it could act as new platform for the immobilization of HRP through noncovalent assembly, the modified HRP catalyzed TMB to oxTMB in the presence of H_2O_2 to produce an electrochemical signal.

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