Electrochemistry Communications 61 (2015) 36–39

Contents lists available at ScienceDirect

Electrochemistry Communications

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

Direct potential resolution and simultaneous detection of cytosine and 5-methylcytosine based on the construction of polypyrrole functionalized graphene nanowall interface

Po Wang [a,b,*], Ping Han [a], Liang Dong [a], Xiangmin Miao [a]

[a] School of Chemistry and Chemical Engineering, Jiangsu Normal University, Xuzhou 221116, China
[b] Department of Chemistry and Biochemistry, FL International University, Miami 33199, United States

ARTICLE INFO

Article history:
Received 17 August 2015
Received in revised form 23 September 2015
Accepted 29 September 2015
Available online 9 October 2015

Keywords:
Potential resolution
Cytosine
Methylated cytosine
Graphene nanowall

ABSTRACT

Herein, an efficient approach was developed for direct electroanalysis of DNA methylation based on the construction of polypyrrole functionalized graphene nanowall interface. Taking advantage of the physicochemical properties of graphene nanowall layer, the mass transport regime of analytes was changed from planar diffusion to thin layer diffusion. As a result, the oxidation signal of 5-methylcytosine (mC) was clearly distinguished from that of cytosine (C) with a direct potential resolution of 184 mV, which paved the way for simultaneous detection with high reliability. Moreover, the proposed method realized the evaluation of DNA methylation level in CpG oligonucleotides in a straightforward, rapid, convenient, label-free and hybridization-free way, suggesting that the system is promising for sensing applications.

1. Introduction

DNA methylation is an important epigenetic modification in human genome that plays a crucial role in the regulation of heritable information [1,2]. The conversion of cytosine (C) to 5-methylcytosine (mC) in promoter CpG islands can induce the change of chromatin structure, and result in the silence of gene transcription [3]. So far, classical methods for the detection of DNA methylation mainly include chromatography-based techniques [4], methylation-sensitive restriction enzyme cleavage assay [5], and methylation-specific polymerase chain reaction (PCR) [6]. The traditional methods are effective in profiling DNA methylation statuses, however, these methods often require time-consuming and complicated procedures [4], or are confined to limited recognition sequences of restriction enzymes [7,8], or are associated with false-positive and significant inaccuracy [9].

Electrochemical approach is advantageous for the detection of DNA owing to its intrinsic attractive properties [10–18]. Paleček and Bartošík presented an elegant review for electrochemistry of nucleic acids, including electrochemical detection of DNA methylation [10]. In 2003, the first electrochemical assay of DNA methylation was realized for the detection of human $p16^{Ink4a}$ gene based on the coupling of DNA electrochemical sensor with linker-PCR [19]. Afterward, the characteristic current signal of mC was selectively obtained by direct labeling of osmium complexation in Okamoto's group, who provided a conceptually new method for investigating DNA methylation [20]. Based on the labeling of electroactive thionine and ferrocene acetic acid, the methylation level of a specific CpG site was innovatively recognized by Cai et al. [21,22]. According to the current signals of labeled CdS and PbS quantum dots, Dai and co-workers realized the profiling of multiple methylation loci at nanomolar concentrations [23]. Recently, Muren and Barton developed an excellent strategy for electrochemical assay of DNA methyltransferase activity based on the signal change of covalently labeled methylene blue redox probe [24]. Interestingly, DNA demethylation can also be monitored at enzyme-based electrochemical biosensors in Ai's group [25,26]. Taking advantage of $[Ru(NH_3)_6]^{3+}$ hybridization indicator coupled with peptide nucleic acid (PNA) probe, we constructed a label-free biosensor for screening of the methylation status of p53 gene [27]. Meanwhile, gene-specific methylation was quantified based on the specific interaction between guanine (G) residues and methylene blue indicator [28]. Furthermore, ferrocenylnaphthalene diimide (FND) and its derivative (F4ND) were synthesized as redox indicators for electrochemical discrimination of methylated and unmethylated genes [29–32].

Electrochemical methods are developed as new intriguing techniques to overcome the limitations of traditional approaches, while labeling or hybridization procedures are commonly needed. In comparison with the analysis performances of electrochemical labeling and hybridization indicator, the direct electrochemical oxidation or reduction of DNA bases is a more straightforward, rapid and convenient

* Corresponding author at: School of Chemistry and Chemical Engineering, Jiangsu Normal University, Xuzhou 221116, China. Tel.: +86 516 83403165; fax: +86 516 83536977.
E-mail addresses: wangpo@jsnu.edu.cn, wangpo@mail.ahnu.edu.cn (P. Wang).

http://dx.doi.org/10.1016/j.elecom.2015.09.025
1388-2481/

approach [33–35]. However, the accurate oxidation currents of pyrimidine bases are difficult to be acquired at traditional solid electrodes. Moreover, it is hard to discriminate the oxidation potentials of C and mC because of their analogous chemical properties. Based on the preparation of nanocarbon film electrode employing electron cyclotron resonance (ECR) sputtering method, Kato et al. firstly acquired the oxidation signals of mC and C with a peak separation of 150 mV [33]. This method provided an innovative idea for exploring DNA methylation, which motivated the voltammetric determination of mC at multiwalled carbon nanotubes film modified electrode [36]. In 2013, Brotons et al. proposed a screen printed graphite electrode platform, which could be used to detect free DNA bases including mC at micromolar concentrations [37]. Fascinatingly, it was revealed that adenine (A), C, mC and G are reducible at Hg electrodes [38–42], while uracil (U) and thymine (T) are not reducible under the same conditions [43]. Inspired by the decreased differential pulse polarography (DPP) reduction peak of DNA resulted from bisulfite treatment [44], Paleček et al. created an excellent approach for electrochemical detection of DNA methylation at nanomolar and subnanomolar levels [43]. According to bisulfite treatment of reducible Cs into nonreducible U residues, DNA methylation was determined quantitatively at hanging mercury drop electrode (HMDE) and solid amalgam electrode (SAE) [43]. In addition, the methylation degrees of Jurkat genomic DNAs were studied based on the electrochemical reduction of C and A using square wave voltammetry [45,46].

In this communication, polypyrrole functionalized graphene nanowalls were constructed for direct potential resolution and simultaneous detection of C and mC. The conductive graphene nanowall layer altered the mass transport regime of analytes from planar diffusion to thin layer diffusion. Consequently, the oxidation peak potentials of C and mC were distinctly discriminated with notable enhancement of peak currents, which confirmed the reliability for simultaneous detection. The proposed system was efficiently applied to the evaluation of DNA methylation level in short CpG oligonucleotides without the need of labeling, hybridization or enzymatic reaction steps.

2. Experimental

Prior to use, a commercial glassy carbon electrode (GCE) was polished successively with 1.0, 0.3 and 0.05 μm Al_2O_3 slurries, rinsed carefully with deionized water, and washed ultrasonically in ethanol and deionized water for 10 min, respectively. The graphene oxide (GO) was sonicated in 0.05 M sodium dodecyl sulfate (SDS) for 1 h for exfoliation and uniform dispersion. Afterward, pyrrole monomer was dissolved into the above solution with the aid of sonication. The GO/PPy composite film modified GCE was prepared by cyclic voltammetry (CV) scanning from −0.2 to 0.8 V for six cycles at 25 mV s^{-1} in 0.05 M SDS solution containing 0.5 mg mL^{-1} GO and 0.05 M pyrrole. The resulting GO/PPy/GCE was then rinsed with deionized water, and transferred into 0.5 M Na_2SO_4 solution for electrochemical reduction of GO, which was performed by CV scanning from 0 to −1.0 V for six cycles at 50 mV s^{-1}. The obtained electrode, described as graphene/PPy/GCE, was rinsed with deionized water, and transferred into 0.1 M pH 7.0 PBS for electrochemical oxidation of PPy at 1.8 V for 4 min. The resulting graphene/PPyox/GCE was finally washed with deionized water to remove co-deposited species, and dried with ultrapure nitrogen. For control experiments, a PPyox/GCE was also prepared with similar working conditions.

3. Results and discussion

3.1. The characterizations of electrode interface

Field emission scanning electron microscope (FE-SEM) was employed to investigate the morphology characteristics of the graphene/PPyox/GCE. As shown in Fig. 1A, a large amount of wrinkled nanostructures were homogeneously and compactly distributed on GCE substrate. These nanostructures were connected with each other, and assembled to form a wall-like network morphology with large surface area. The resulting nanowalls were perpendicular to the substrate surface, and oriented toward outside of the deposited film. The average thickness of the nanowalls was estimated to be about 14 nm, which was evidently larger than the size of graphene, indicating that the oriented nanowalls were graphenes enwrapped within polypyrrole, instead of graphenes themselves. It should be noted that the vertical graphene nanowalls could remarkably improve the roughness and edge-plane-like defective site density of the electrode interface, which was expectable for electroanalysis applications.

The element and composition informations of the as-prepared graphene nanowall were tested by X-ray photoelectron spectroscopy (XPS). As presented in Fig. 1B, the N1s peak located at 400.2 eV validated the electropolymerization of pyrrole monomer onto the electrode surface, while the S2p signal at 168.5 eV demonstrated the presence of SDS in the nanocomposite. A possible mechanism for the formation of graphene nanowall was described as follows. Based on previous study [47], SDS was easy to be adsorbed at the surface of graphene via van der Waals' force, which could promote the uniform dispersion of graphene in the mixed system. In the process of CV scan, the negatively charged SDS-graphene nanocomposite would orient toward the electrode owing to the presence of electric field [48], resulting in the directional assembly of graphene on GCE. As soon as the electrode was applied with higher potential, the pyrrole monomers were polymerized

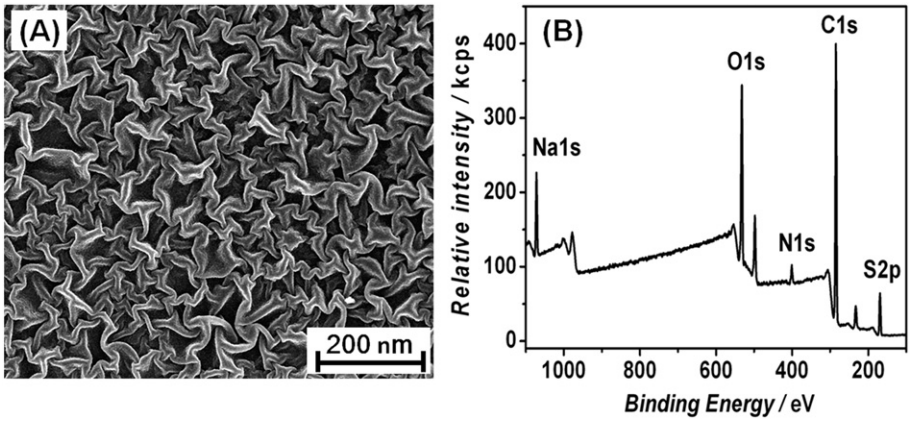

Fig. 1. (A) FE-SEM image of the graphene/PPyox/GCE. (B) XPS spectrum of the as-prepared graphene nanowalls.