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Sensitive electrogenerated chemiluminescence biosensing method for the determination of DNA hydroxymethylation based on $Ru(bpy)_3^{2+}$-doped silica nanoparticles labeling and MoS_2-poly(acrylic acid) nanosheets modified electrode

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ABSTRACT

5-Hydroxymethylcytosine (5-hmC), an oxidation product of 5-mC (5-methylcytosine), is presented in DNA of most mammalian cells and play an important role in the alteration of cancer-related genes. Herein, a sensitive electrogenerated chemiluminescence (ECL) biosensing method for the determination of 5-hmC in DNA (5-hmC DNA) was established on the basis of chemical modification and nanomaterial amplification. First, electrochemically reduced molybdenum disulfide-poly(acrylic acid) (rMoS_2-PAA) nanosheets were used to modify glassy carbon electrode (GCE) to form an ECL biosensing electrode (rMoS_2-PAA/GCE) which has large accessible surface area to immobilize more DNA. Then, a capture probe with amino group was hybridized with the target 5-hmC DNA and immobilized on the surface of rMoS_2-PAA/GCE via amido bond. When cysteamine was introduced, the M.HhaI methyltransferase (M.HhaI) was used as specific recognition element to replace the hydroxyl group of 5-hmC by thiol and generated the amine-derivated DNA. Finally, surface chemically activated $Ru(bpy)_3^{2+}$-doped silica ($Ru@SiO_2$) nanoparticles, carriers of ECL reagents, were employed as signal amplification unit which covalently bonded to the amine-derivated DNA resulting in an increased ECL intensity. The increased ECL intensity was linearity to the 5-hmC DNA concentration in a range from 5.0×10^{-14} M to 1.0×10^{-11} M, with a lower detection limit of 1.2×10^{-14} M. Besides, the proposed method also displayed a good selectivity to 5-hmC in the presence of 5-C and 5-mC. Moreover, the developed biosensing method was successfully employed to monitor human urine sample.

1. Introduction

5-Hydroxymethylcytosine (5-hmC) is a naturally occurring nucleobase which abundantly existed in neurons and embryonic stem cells [1,2]. 5-methylcytosine (5-mC, the fifth base of gene) is oxidized to 5-hydroxymethylcytosine (5-hmC), resulting in DNA hydroxymethylation (5-hmC DNA). 5-hmC is not simply a vital DNA demethylation intermediate in the progress of replication-independent, but a genuine epigenetic mark that play key roles in reprogramming [3,4] and cancer [5,6]. The studies on cancer found that loss of 5-hmC occurs in different human cancers, such as breast, gastric, kidney, liver, lung, pancreatic and prostate cancers [7–11]. Thus, decreased 5-hmC level in cancer cells may also be an urgent indicator in cancer diagnosis [12,13]. Therefore, the development of novel, reliable, simple, and sensitive analytical technologies for detection of DNA hydroxymethylation is an important and in urgent need.

To date, various strategies have been constructed for the detection of DNA hydroxymethylation, including liquid chromatography-mass spectrometry (HPLC-MS) [14], fluorescence microscopy [15], photoelectrochemistry [16], optical microtoroid resonant cavity biosensing method [17], electrochemical method [18], electrogenerated chemiluminescence (ECL) [19]. Though many alternatives for DNA hydroxymethylation detection, a new method with improved sensitivity is still needed for the detection of DNA hydroxymethylation.

ECL technique has received considerable attention and played an important role in biomolecules detection, due to its high sensitivity,

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controllability, and simplified operation [20]. Yang et al. proposed an aptamer-based biosensor array for the detection of multiple AMI biomarkers via a versatile signal probe [21]. Feng et al. presented a surface-confined DNA assembly amplification ECL biosensor for DNA detection based on DNA nanostructural scaffold which achieved a satisfying sensitivity [22]. Our group also established several ECL biosensing methods for the discrimination and detection of DNA hydroxymethylation [19,23,24]. Though these methods are available for DNA hydroxymethylation detection, there are still limitations existed, such as the incompletely oxidation of 5-hmC, high background of ECL biosensing electrode. Thus, an effective method is still required for the detection of DNA hydroxymethylation.

As we know, the nanomaterials were used as a promoter to enhance ECL signal and improve the performance of the biosensing electrode [25]. One-pot synthesized the $Ru(bpy)_3^{2+}$-doped silica ($Ru@SiO_2$) was used as an ideal tag in bioanalysis, because of its easy preparation, effectively conjugating to the desired biomolecules and promoting the sensitivity of ECL detection by immobilizing a high concentration of $Ru(bpy)_3^{2+}$ [26–28]. Besides, molybdenum disulfide (MoS_2), a promising layer two-dimensional nanosheet, has been widely applied in biosensing methods for small molecules, DNA, RNA, protein detection [29–32]. Owing to the excellent electron transfer rate and large accessible surfaces area, MoS_2 was used as the substrate to fabricate the biosensing electrode for DNA hydroxymethylation detection in this work.

Herein, a sensitive ECL biosensing method is proposed for DNA hydroxymethylation detection. The detection mechanism of the ECL biosensing method is presented in Fig. 1. First, electrochemically reduced molybdenum disulfide-poly(acrylic acid) ($rMoS_2$-PAA) nanosheets were used to modify glassy carbon electrode (GCE) to form an ECL biosensing electrode ($rMoS_2$-PAA/GCE) which has large accessible surface area to immobilize more DNA. Then, amino-functionalized double-stranded DNA (capture probe & 5-hmC DNA, S1 & S2) was immobilized on the surface of $rMoS_2$-PAA/GCE via amido bond. When cysteamine was introduced, the M.HhaI methyltransferase (M.HhaI) was used as specific recognition element to replace the hydroxyl group of 5-hmC by thiol and generated the amine-derived DNA [33]. Finally, surface chemically activated $Ru(bpy)_3^{2+}$-doped silica ($Ru@SiO_2$) nanoparticles, carriers of ECL reagents, were employed as signal amplification unit. They covalently bonded to the amine-derived DNA resulting in an increased ECL intensity. Consequently, the target 5-hmC DNA concentration can be indirectly evaluated by measuring the ECL intensity. The characteristics of the ECL method were explored, and the detection performance for DNA hydroxymethylation was stated in the following work.

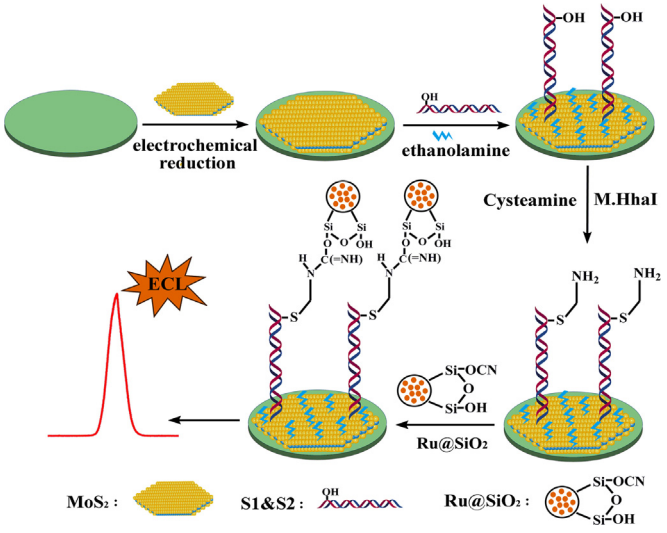

Fig. 1. Schematic illustration of ECL biosensing assay for DNA hydroxymethylation.

2. Experimental

2.1. Reagents and apparatus

The materials, apparatus and the preparation of exfoliated MoS_2 nanosheets and $Ru@SiO_2$ nanoparticles are presented in the Supplementary Information. The encapsulated efficiency of $Ru@SiO_2$ nanoparticles was calculated to be about 8.6 wt% by deducting the fluorescence intensity of the supernate from that of 0.5 mg mL^{-1} $Ru(bpy)_3^{2+}$ solution. In addition, according to the literature [34], the total number of $Ru(bpy)_3^{2+}$ in per $Ru@SiO_2$ nanoparticle was estimated to be 3.4×10^5.

2.2. Fabrication of biosensing electrode

A glassy carbon electrode (GCE, Φ = 3.0 mm) was polished with 0.3, 0.05 μm alumina slurry and rinsed thoroughly with water. 0.5% PAA were mixed with MoS_2 suspension (MoS_2 final concentration is 0.5 mg mL^{-1}) and then sonicated for 10 min to prepare MoS_2-PAA mixture. Next, 10 μL MoS_2-PAA mixture was dropped onto the cleaned GCE and electrochemically reduced to form an ECL biosensing electrode ($rMoS_2$-PAA/GCE). After that, the electrode was dipped into 50 μL 400 mM N-(3-Dimethylaminepropyl)-N-ethylcarbodiimide hydrochloride (EDC) and 100 mM N-hydroxysuccinimide (NHS) solution at 37 °C for 1 h to active carboxylic group of $rMoS_2$-PAA mixture. The resulting $rMoS_2$-PAA/GCE was then thoroughly washed and dried in the air.

2.3. Electrochemical measurement

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) analyses were performed on a CHI 660 electrochemical workstation (Shanghai Chenhua Instruments Co. Ltd. China, http://www.chinstr.com). CV, EIS measurements were performed in the presence of 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) containing 0.1 M KCl. The CV measurement was performed from − 0.1–0.6 V at a scan rate of 50 mV s^{-1}. The EIS measurement was performed from 0.1 Hz to 100 kHz at 5.0 mV wave amplitude and at an electrode potential of 0.23 V. The conventional three-electrode cell was used, which included a platinum wire as a counter electrode, an Ag/AgCl (sat. KCl) as reference electrode and a modified GCE ($rMoS_2$-PAA/GCE, S2&S1/$rMoS_2$-PAA/GCE, NH_2-S2&S1/$rMoS_2$-PAA/GCE, $Ru@SiO_2$/NH_2-S2&S1/$rMoS_2$-PAA/GCE) or bare GCE as working electrode.

2.4. ECL measurement

For detection of DNA hydroxymethylation, $rMoS_2$-PAA/GCE was soaked in 50 μL different concentration of S1&S2 hybridization buffer at 37 °C for 90 min (the obtained electrode named as S2&S1/$rMoS_2$-PAA/GCE). After blocking with ethanolamine for 30 min at 37 °C, 10 μL 100 U mL^{-1} M.HhaI reaction solution containing 50 mM sodiumacetate (pH 6.5), 0.2 mg mL^{-1} BSA, 5% glycerol and 13 mM cysteamine were coated on the prepared electrode at 37 °C for 60 min (the resulted electrode named as NH_2-S2&S1/$rMoS_2$-PAA/GCE). Finally, the modified electrode was droped with 10 μL 5 mg mL^{-1} surface chemically activated $Ru@SiO_2$ nanoparticles at 37 °C for 120 min (the resulted electrode named as $Ru@SiO_2$/NH_2-S2&S1/$rMoS_2$-PAA/GCE). The electrode was washed with 0.1 M phosphate buffered saline (PBS) to remove the nonspecific absorption of the $Ru@SiO_2$ nanoparticles before ECL measurements. ECL measurements were carried out by applying the potential range of 0 ~ 1.4 V (vs. Ag/AgCl) at a scanning rate of 50 mV s^{-1} in 0.1 M PBS (pH 7.4) and 50 mM TPA. To illustrate the sensitivity of proposed method, $Ru@SiO_2$ was displaced by tris(2,2′-