Sensors and Actuators B 220 (2015) 101–106

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Electrochemical biosensor for DNA methyltransferase detection based on DpnI digestion triggering the formation of G-quadruplex DNAzymes

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ARTICLE INFO

Article history:
Received 22 January 2015
Received in revised form 13 May 2015
Accepted 20 May 2015
Available online 3 June 2015

Keywords:
DNA methyltransferase
G-quadruplex DNAzymes
Electrochemical biosensor
Inhibitor

ABSTRACT

Aberrant DNA methylation, which is caused by the abnormal level of DNA methyltransferase (MTase), has been considered associated with a growing number of human diseases. Although there are various methods paying close attention to DNA methyltransferase (MTase) detection, most of them are generally complex and expensive. Here, a simple electrochemical strategy for sensitive detection of DNA methyltransferase (MTase) and inhibitor screening based on DpnI digestion triggering the formation of G-quadruplex DNAzymes has been developed. In this paper, a probe richness of guanine (G) was first self-assembled on the surface of the electrode through Au–S bond and then hybridized with the complementary DNA. Without DNA methylation, G-quadruplex DNAzymes cannot be formed due to the double helix structure and a weak electrochemical response can be observed. On the contrary, an obvious enhancement of the electrochemical response can be achieved after the cleavage of the methylated double-strand DNA by DpnI since G-quadruplex DNAzymes can be obtained, which catalyze the oxidation of hydroquinone by H_2O_2 with the assistance of the cofactor hemin. This method is under a detection limit of 0.96 U/mL and can monitor the change of DNA methylation level selectively. Moreover, RG108 was selected as a representative inhibitor for studying the inhibition activity of DNA MTase.

1. Introduction

DNA methylation, one of the essential epigenetics events, plays a major role in the regulation of gene expression [1], gene transcription [2] and embryogenesis [3] in all prokaryotes, eukaryotes and mammals. This significant biological activity is under the responsibility of methyltransferase (MTase), which catalyzes a transfer of a methyl group from *S*-adenosyl-*L*-methionine (SAM) to adenine or cytosine [4] in the target DNA. It is indicated that abnormal level of DNA MTase can cause aberrant DNA methylation which is closely associated with pathogenic mechanisms to human, especially cancer [5,6]. Hence, detecting the change of DNA MTase level is essential for future genetic diseases diagnosis.

So far, various methods have been proposed for detection of DNA MTase, mainly including molecular imprinting method [7], combined bisulfite restriction analysis (COBRA) [8], methylation-specific PCR (MSP) [9], methylation-sensitive single nucleotide primer extension (Ms-SNuPE) [10], high performance liquid chromatography (HPLC) [11], immunochemical [12]. Although each method has its own advantages, most of them are time-consuming and tedious. Recently, some methods are developed to avoid the shortcomings of the traditional ways, such as methylight [13] and colorimetric approaches [14]. However, these methods also have the disadvantages of expensive and complex. Therefore, a simple, efficient and sensitive approach for DNA MTase detection is still a need.

Electrochemical technology has been extensively used for DNA analysis [15,16]. It has a series of promising abilities such as simple, inexpensive and highly sensitive to study DNA MTase detection. For instance, Li et al. [17] presented a DNA MTase activity detection method based on the voltammetric response of the electroactive label thionine and graphene oxide. Xu et al. [18] developed an electrochemical biosensor to detect the activity of DNA MTase which used the streptavidin-alkaline phosphatase to convert 1-naphthyl phosphate into 1-naphthol which can be electrochemically oxidized. Another method based on the electrochemical immunosensing platform has also been developed by our group, which used the horseradish peroxidase labeled goat

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http://dx.doi.org/10.1016/j.snb.2015.05.058
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antimouse IgG to catalyze hydroquinone oxidation by H_2O_2 and monitor the activity of DNA MTase. These methods do not need tedious process or large amount of time but require fussy assembly of nanoparticle and rigorous condition for enzyme reaction. So it is toujours valuable to develop a new electrochemical method for detection of DNA MTase.

G-quarduplex is a G-rich oligonucleotide sequence which can fold into a specific structure in the presence of Na^+ or K^+ [19]. It can be formed by a long or parts of short single-stranded DNA [20,21]. Upon complexation with hemin, the G-quarduplex DNAzymes can be achieved which have an enhanced horseradish peroxidase (HRP) -mimicking activity [22]. The application of the DNAzymes is abroad owing to their high stability and sensitivity. In our paper, the strategy consists primarily of a probe richness of guanine (G) and DNA MTase-restriction endonuclease reaction system, composed of Dam MTase and DpnI. After catalyzing by G-quarduplex DNAzymes, the change of DNA methylation level can be monitored by the differential pulse voltammetry (DPV) signal of benzoquinone. In addition, this method is also suitable for the screening of MTase inhibitors.

2. Experimental

2.1. Reagents and materials

6-Mercapto-1-hexanol (MCH) was purchased from Alfa Aesar (Lancashire, England). Hydrogen tetrachloroaurate trihydrate ($HAuCl_4{\cdot}3H_2O$), tris (hydroxymethyl) aminomethane (Tris), tris (2-carboxyethyl) phosphine hydrochloride (TCEP) and hemin were purchased from Aladdin (Shanghai, China). Dam MTase and restriction endonuclease DpnI were respectively supplied by New England Biolabs (Ipswich, MA) and Fermentas (MD, USA). According to the supplier, Dam MTase was stored at −20 °C in a buffer containing 50 mM KCl, 50 mM Tris–HCl (pH 7.5), 10 mM EDTA, 1 mM dithiothreitol, 200 μg/mL BSA and 50% glycerol. Restriction endonuclease DpnI was stored in a buffer containing 10 mM Tris–HCl (pH 7.4), 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 500 μg/mL BSA, 50% Glycerol at −20 °C in the refrigerator. All of the reagents were analytically pure and the solutions were prepared by double distilled water after high pressure steam sterilization.

DNA was obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China) and stored in the TE buffer containing 10 mM Tris–HCl (pH 8.0) and 1 mM EDTA at −20 °C in the refrigerator. The probe DNA is modified at the 5-end with a thiol group (S1: 5′-HS-$(CH_2)_6$-TTC TAA TCT GAA GCG GGT AGG GGA TCT TGG G-3′). The complementary target (S2) has the following sequence: 5′-CCC AAG ATC CCC-3′.

Electrochemical experiments were carried out on a CHI660C electrochemical workstation (Austin, USA). A glassy carbon electrode (GCE), a saturated calomel electrode (SCE) and a platinum wire are used as the working electrode, reference electrode and the auxiliary electrode, respectively.

2.2. Deposition of gold nanoparticles (AuNPs)

The GCE was first polished with alumina powder (30 nm) to a mirror-like surface. And then the electrode was washed with double distilled water, anhydrous ethanol and double distilled water by ultrasonication for 3 min, respectively. Next, the gold nanoparticles (AuNPs) were electrodeposited onto GCE surface in a 3 mM $HAuCl_4$ solution containing 0.1 M KNO_3 using the amperometry technique at −0.2 V for 200 s according to previous report [23]. After thoroughly rinsed with double distilled water, the obtained AuNPs/GCE was ready for DNA immobilization and characterized by SEM (Fig. S1 in Supplementary Materials).

2.3. Immobilization and hybridization of probe DNA

In order to assemble probe DNA, 5 μL of probe immobilization buffer (10 mM Tris–HCl, 1.0 mM EDTA, 1.0 M NaCl and 1.0 mM TCEP, pH 7.0) containing 5.0×10^{-7} M DNA S1 was dripped on the surface of AuNPs/GCE. And then the electrode was incubated for 2 h at drippy condition for the formation of Au–S bond between the thiol group at the 5′-end of DNA S1 and AuNPs, following by rinsing the electrode with 10 mM of Tris–HCl buffer (pH 7.0). After that, 5 μL of Tris–HCl (10 mM) containing 1.65×10^{-6} M MCH was dripped on the electrode surface. The –SH moiety of MCH would react with AuNPs to form Au–S bond which could keep a good orientation of probe DNA. One hour later, the electrode (named as ssDNA/AuNPs/GCE) was thoroughly rinsed with Tris–HCl buffer (10 mM, pH 7.0).

Hybridization was conducted at 37 °C by incubating ssDNA/AuNPs/GCE with 5 μL hybridization buffer (10 mM Tris–HCl, 1.0 mM EDTA and 1.0 M NaCl, pH 7.0) containing DNA S2 (5.0×10^{-7} M) for 2 h. Finally, the electrode was rinsed with vast Tris–HCl buffer containing 0.1 M NaCl and noted as dsDNA/AuNPs/GCE.

2.4. Methylation of the CpG and cleavage

The methylation of S1/S2 hybrid was performed at 37 °C for different time (0–140 min) by dropping 5 μL of 50 mM Tris–HCl buffer (pH 7.5) containing 80 μM SAM, 10 mM EDTA, 5 mM 2-mercaptoethanol and various concentration of Dam MTase (0–60 U/mL). After methylation, the electrode was thoroughly washed and transferred into 20 mM Tris-acetate buffer (pH 7.9) containing 20 U/mL DpnI, 50 mM Potassium acetate, 10 mM magnesium acetate and 100 μg/mL BSA for cleavage. Then the electrode was incubated at 37 °C for 2 h and washed thoroughly again.

2.5. Inhibition activity of Dam MTase

To study the inhibition effect of the anticancer drug on the Dam MTase activity, the methylation of S1/S2 hybrid was performed at 37 °C for 2 h in 50 mM Tris–HCl buffer (pH 7.5) containing 40 U/mL of Dam MTase, 80 μM SAM, 10 mM EDTA, 5 mM 2-mercaptoethanol and various concentration of RG108 (0–1000 nM). The inhibition efficiency (%) is estimated as follows:

$$\text{Inhibition}\,(\%) = \frac{I_2 - I_3}{I_2 - I_1} \times 100\%$$

where I_1 was the current of the electrode treated successively with Dam MTase and DpnI, I_2 was current of the electrode treated successively with Dam MTase, DpnI and hemin, and I_3 was the inhibited current.

2.6. Electrochemical determination

For forming the G-quadruplex, 5 μL of 20 mM Tris–HCl buffer (pH 7.4) containing 40 mM KCl, 200 mM NaCl, 6% Triton-100 (volume fraction) was dripped on the surface of the electrode. After reacting for 1 h, 5 μL of hemin was added and the electrode was incubated at 37 °C for another hour.

Differential pulse voltammetry (DPV) was performed in 10 mL of 0.1 M PBS (pH 7.40) containing 20 mM KCl, 50 μM H_2O_2 and 50 μM hydroquinone. DPV signals were measured using a potential step of 4 mV, pulse amplitude of 50 mV, pulse width of 0.05 s, sample width of 0.0167 s, pulse period of 0.2 s and quiet time of 2 s.