Analytica Chimica Acta 934 (2016) 66–71

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Electrochemical DNA sensor-based strategy for sensitive detection of DNA demethylation and DNA demethylase activity

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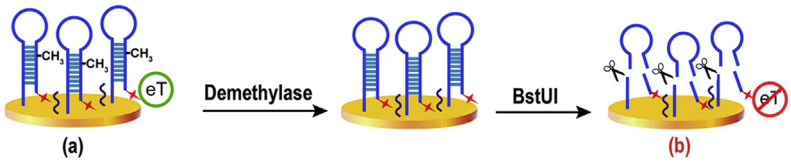
HIGHLIGHTS

- Detect DNA demethylase activity by electrochemical DNA sensor strategy.
- The principle relies on electrochemical signal changes of FcA redox label.
- Combine the DNA demethylation and the BstUI endonuclease digestion.
- A high sensitivity with low detection limit of 0.17 ng/mL of DNA demethylase.
- The assay can be used for the related molecular diagnostics and drug screening.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 19 March 2016
Received in revised form
11 June 2016
Accepted 21 June 2016
Available online 27 June 2016

Keywords:
Electrochemical DNA sensor
DNA demethylation
DNA demethylase
Endonuclease
Redox label

ABSTRACT

DNA demethylation and demethylase activity play important roles in DNA self-repair, and their detection is key to early diagnosis of fatal diseases. Herein, a facile electrochemical DNA (E-DNA) sensor was developed for the sensitive detection of DNA demethylation and demethylase activity based on an enzyme cleavage strategy. The thiol modified hemi-methylated hairpin probe DNA (pDNA) was self-assembled on a Au electrode surface through the formation of Au–S bonds. The hemi-methylated pDNA served as the substrate of DNA demethylase (using methyl-CpG-binding domain protein 2 (MBD2) as an example). Following demethylation, the hairpin stem was then recognized and cleaved by BstUI endonuclease. The ferrocene carboxylic acid (FcA)-tagged pDNA strands were released into the buffer solution from the electrode surface, resulting in a significant decrease of electrochemical signal and providing a means to observe DNA demethylation. The activity of DNA demethylase was analyzed in the concentration ranging from 0.5 to 500 ng mL^{-1} with a limit of detection as low as 0.17 ng mL^{-1}. With high specificity and sensitivity, rapid response, and low cost, this simple E-DNA sensor provides a unique platform for the sensitive detection of DNA demethylation, DNA demethylase activity, and related molecular diagnostics and drug screening.

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1. Introduction

As a mark of transcriptional silencing, DNA methylation plays important roles in the regulation of gene transcription,

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http://dx.doi.org/10.1016/j.aca.2016.06.037
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development of eukaryotes, and cellular differentiation, as well as the pathogenesis of serious human diseases [1–4]. Recent breakthroughs have shown that the methylation state of DNA can be dynamically removed during early mammalian development by active demethylation [5–8]. As the opposite of DNA methylation, during the DNA demethylation process, the patterns of DNA methylation can be reprogrammed and removed by DNA demethylase. For example, the methyl-CpG-binding domain protein (MBD2) has been reported to possess demethylase activity and can remove the methyl group from 5-methyl cytosine [9–13]. DNA active demethylation has been reported to play a crucial role in the epigenetic regulation of genes. Dysregulation of DNA demethylation and DNA demethylase is involved in many serious diseases, including cancers, imprinting-related diseases, and psychiatric disorders. Hence, sensitive detection of DNA demethylation and demethylase activity has attracted intense interest in the past decades for both biochemical and clinical research. Although biosensors for DNA methylation and methyltransferases have been wildly reported [14–19], relatively little attention has been focused on DNA demethylation and DNA demethylase assay [10–13].

In order to meet these objectives, various methods have been developed for the assay of DNA demethylase activity over the past years, including fluorescence [12], surface enhanced Raman scattering [13] and electrochemical methods [10,11]. Although several electrochemical methods have been used in the detection of DNA demethylation and DNA demethylase activity, they usually used other enzymes to amplify the signal. For example, Zhou et al.'s enzymatic signal amplification method [10] involved conventional DNA demethylase treatment of the biotin tagged hemi-methylated DNA, followed by capture streptavidin tagged alkaline phosphatase (SA-ALP) to catalyze the reaction. The dual enzyme digestion signal amplification method by Yin et al. [11] used BstUI to digest the demethylated DNA, which was further cleaved by Exonuclease III. These two methods are highly sensitive and selective, but relatively expensive and require multiple separation and wash steps, which restrict their utility for routine assays. In addition, if both BstUI and Exonuclease III are in the same system, they may interfere with each other. Thus, it is still highly desirable to develop a facile method that can provide a simple and practical means for sensitive and selective detection of DNA demethylation and demethylase activity.

Over the past decade, a simple electrochemical DNA (E-DNA) technique has drawn considerable attention for the sensitive and selective detection of different sequence-specific nucleic acids [15–17,20]. A typical E-DNA sensor is based on the conformational change of a redox-labeled DNA probe in the presence of the target sequence. This change alters the distance between the redox label and the electrode surface and generates either increased or decreased current response for detection. The distinct advantages of the electrochemical DNA method, including high sensitivity, simple and inexpensive instrumentation requirements, rapid response, low fabrication cost, and portability, make E-DNA a suitable platform for the detection of DNA demethylase.

In this research, we developed an E-DNA sensor for sensitive detection of DNA demethylation and demethylase activity based on an enzyme cleavage strategy. As shown in Fig. 1, the hairpin probe DNA (pDNA), which is hemi-methylated at a cytosine in the stem region, is self-assembled on the Au electrode surface through the formation of a Au–S bond on the 5′ end. The ferrocene carboxylic acid (FcA) tag on the 3′ end which is in close proximity to the gold electrode produces a large faradaic current. In the absence of demethylases, cleavage by the BstUI endonuclease is blocked by cytosine methylation, thus preserving efficient electron transfer between the FcA tags and the sensor surface. However, when the hemi-methylated pDNA is demethylated by MBD2, the demethylation site on the symmetrical duplex 5′-CGCG-3′ sequence of the hairpin stem can be recognized and cleaved by BstUI endonuclease [21]. After digestion by BstUI, the FcA tags are released into the buffer solution from the electrode surface, resulting in a significant decrease or disappearance of the electrochemical signal. Therefore, the electrochemical signal is related to the demethylation level and demethylase activity. Based on this strategy, the DNA demethylation and demethylase activity can be directly detected from the magnitude of the electrochemical signal. This simple E-DNA sensor shows distinct advantages in its high sensitivity, specificity, facile operation, low cost and rapid response, making it a promising platform for detecting DNA demethylation, demethylase activity, and related molecular diagnostics and drug screening.

2. Experimental section

2.1. Materials and reagents

Tris (hydroxymethyl) aminomethane (Tris), human serum albumin (HSA), Tris (2-carboxyethyl) phosphine hydrochloride (TCEP, 98%), 6-mercapto-1-hexanol (MCH), Ferrocene carboxylic acid (FcA, 98%), *N*-(3-dimethylaminopropyl)-N′- ethylcarbodiimide (EDC) and *N*-hydroxysuccinimide (NHS, 98%) were purchased from Sigma-Aldrich Co., Ltd (St. Louis, MO, USA). The other chemicals and reagents were of analytical grade, and obtained from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). All solutions were prepared with ultrapure water, which was obtained from a Millipore Milli-Q purification system (Millipore, Spain) and had an electric resistance >18.3 MΩ. The washing solution was 10 mM Tris-HCl buffer (pH 7.4).

DNA demethylase (methyl-CpG-binding domain protein 2, MBD2) and MBD1, MBD4, and MeCP2 were obtained from Epigentek Group Inc (Farmingdale, NY, USA). Restriction endonuclease BstUI was from New England Biolabs Ltd (Ipswich, MA, USA).

The thiol-modified hemi-methylated hairpin pDNA strands were synthesized by Sangon Biotech Co., Ltd (Shanghai, China) and purified with high-performance liquid chromatography. Their base sequences are listed as follows:

5′–SH–$(CH_2)_6$-TTCTCTTCCTCTGTGCGCTCT*CACGCG*TGTTCTAT CAC^m*GCG*TG-NH_2-3′, in which the two underlined complementary sequences make the probe form a hairpin structure. The CGCG sequence in italics is the recognition site of BstUI endonuclease, and hemi-methylation of this site can block the BstUI cleavage reaction. All DNA solutions were prepared using 10 mM Tris-HCl buffer (pH 7.4).

2.2. Apparatus

Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry were used for characterization of the processes of this assay. EIS was performed at an amplitude of 0.005 V in the presence of 5 mM $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ solution containing 0.5 M KCl at a 0 V bias potential against the open circuit potential in the frequency range 100 kHz–100 mHz. Typical cyclic voltammograms of the electrode were measured at 50 mV s^{-1} in 0.1 M acetate buffer (pH 5.0). The pH value of electrolyte was determined by a 320-S acidity meter (Mettler-Toledo, Switzerland).

All electrochemical measurements were performed on an Autolab PGSTAT302N electrochemical station (Metrohm) in a conventional three-electrode electrochemical cell with the gold electrode as working electrode, and a saturated calomel electrode (SCE) and a platinum wire as reference electrode and auxiliary electrode, respectively. Prior to measurements, all solutions were degassed by flowing pure N_2 for 10 min and kept under a N_2 ambient throughout the measurement.

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