Journal of Electroanalytical Chemistry 814 (2018) 144–152

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

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

A simple and universal electrochemical assay for sensitive detection of DNA methylation, methyltransferase activity and screening of inhibitors

Jing Bao[a,1], Xintong Geng[a,1], Changjun Hou[a,*], Yanan Zhao[a], Danqun Huo[b,*], You Wang[a], Zhaonan Wang[a], Yan Zeng[a], Mei Yang[a], Huanbao Fa[c]

[a] Key Laboratory of Biorheological Science and Technology (Chongqing University), Ministry of Education, College of Bioengineering, Chongqing University, Chongqing 400044, PR China

[b] Liquor Making Biology Technology and Application of Key Laboratory of Sichuan Province, College of Bioengineering, Sichuan University of Science and Engineering, Zigong 643000, PR China

[c] College of Chemistry and Chemical Engineering, Chongqing University, Chongqing 400044, PR China

ARTICLE INFO

Keywords:
Electrochemical biosensor
DNA methylation
Methyltransferase
Inhibitors

ABSTRACT

Many studies have confirmed that DNA methylation is highly correlated with the occurrence and development of various diseases including cancers. In this work, we developed a simple, sensitive, selective, and universal electrochemical biosensor for detection of DNA methylation and assay of DNA methyltransferase (MTase) activity using M.SssI MTase as an example. The thiolated single-stranded DNA S1 was self-assembled on the surface of gold nanoparticles deposition modified glassy carbon electrode via Au—S bonding, then hybridization between the DNA S1 and its complementary DNA S2 formed a double-stranded target sequence for both M.SssI MTase and restriction endonuclease *Hpa*II. HpaII could not cleave the target sequence after it was methylated by M.SssI MTase, while the sequence without methylation could be cleaved. Here, we used methylene blue (MB) as electrochemical indicator. The electrochemical signal of MB increased linearly with increasing M.SssI MTase concentration from 0.5 to 25 U/mL and from 25 to 400 U/mL with a detection limit of 0.04 U/mL. Moreover, screening of M.SssI MTase inhibitors 5-azacytidine (5-Aza) and 5-Aza-2′-deoxycytidine (5-Aza-dC) were successfully investigated using the fabricated electrochemical biosensor and showed that the two classic drugs could both inhibit the M.SssI MTase activity with the IC_{50} of 2.8 μM and 0.37 μM, respectively, indicating potential application in discovery of new anticancer drugs.

1. Introduction

Epigenetics, a branch of biology, pertains to the heritable changes in gene expression without altering the underlying DNA sequence. There are three major ways to regulate the expression of a gene which contains DNA methylation, histone modifications, and RNA-associated silencing [1]. Among them, DNA methylation is the best known epigenetic marker which plays a crucial role in many biological processes, such as cellular development, gene expression and regulation, and is involved in the maintenance of genomic integrity and tumorigenesis [2–4]. Numerous studies have proved that aberrant DNA methylation is correlated with various diseases including cancers. For example, the methylation of *Homo sapiens p53* gene has been confirmed in a wide variety of cancers, such as lung cancer, breast cancer, colon cancer, bone cancer, ovaries cancer, and bladder cancer [5]. Thus, the detection of DNA methylation is a convenient tool for cancer diagnosis. The DNA methylation process is usually caused by DNA methyltransferase (MTase), which is able to transfer a methyl group from *S*-adenosyl-L-methionine (SAM) to cytosine or adenine at particular sequences [6], so the level of DNA methylation is closely related to the activity of DNA MTase. In addition, the aberrant activity of DNA MTase is associated with the pathogenesis of many cancers, which provides a predictive biomarker and potential therapeutic targets for interrelated cases. Therefore, it is of great significance to develop simple, selective, and sensitive strategies for DNA MTase activity assays.

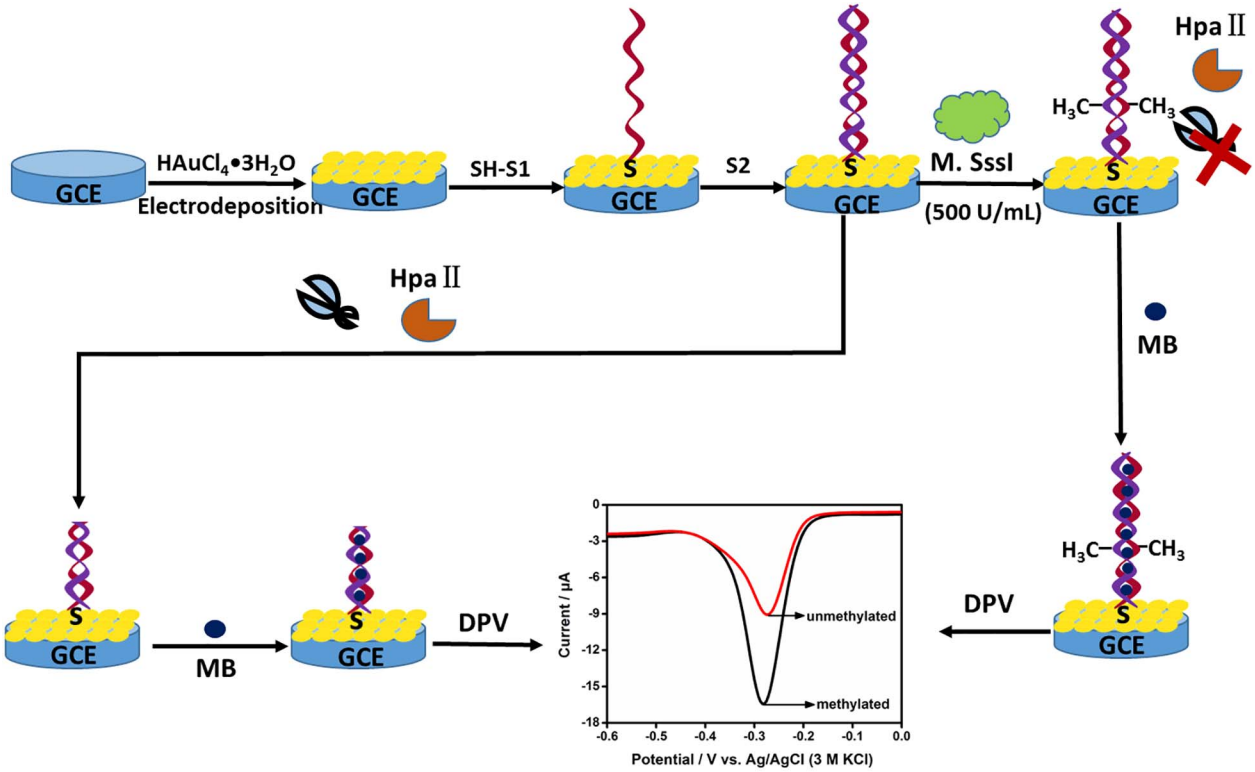
Thanks to continuous efforts over the last decade, a variety of assays have been established for investigating DNA methylation and DNA MTase activity, including bisulfite methods [7–9], polymerase chain reaction (PCR) [10], fluorescence-based biosensors [11,12], colorimetric methods [13], microarray based DNA methylation profiling [14], surface enhanced Raman spectroscopy-based assays [15], surface plasma resonance (SPR) [16], high-performance liquid chromatography

* Corresponding authors.
E-mail addresses: houcj@cqu.edu.cn (C. Hou), huodq@cqu.edu.cn (D. Huo).
[1] Those authors contribute equally to this work.

https://doi.org/10.1016/j.jelechem.2018.02.060
Received 3 December 2017; Received in revised form 2 February 2018; Accepted 26 February 2018
Available online 28 February 2018
1572-6657/

(HPLC) [17], photo-electrochemistry methods [18,19], electrochemiluminescence methods [20,21] and electrochemistry methods [22–25]. Among these methods, electrochemical methods have attracted more and more attention for exhibiting unique merits of time-saving, simple operation, low production cost, high sensitivity, and selectivity. For instance, Su et al. designed a signal-on electrochemical sensor for the detection of DNA MTase activity based on a hairpin DNA probe with limits of detection of 0.07 U/mL [26]. Wei and Ma developed a solution-phase electrochemical method for DNA methylation detection based on an ITO microelectrode chip [27]. Cai's group reported an electrochemical biosensor for the detection of the genomic DNA methylation level based on the electro-active label (ferrocenecarboxylic acid, FcA) [28]. Wang et al. used horseradish peroxidase labeled goat anti-mouse IgG (HRP-IgG) to catalyze hydroquinone oxidation by H_2O_2 and detect the DNA methylation and the activity of DNA MTase with a limit of detection as low as 0.1 U/mL [29]. Good performances have been realized through some of the above-mentioned methods, however, tedious labeling procedures or multistep steps are involved in them. Therefore, further efforts are needed in the development of simple, convenient, and economical methods for the detection of DNA methylation.

In this study, we developed a simple, sensitive and universal electrochemical approach for detecting DNA methylation and assay of M.SssI MTase activity based on DNA-AuNPs amplification. The schematic diagram of this approach is shown in Scheme 1. We all know that AuNPs have been widely used in the region of electrochemical biosensors for the detection of glucose, nucleic acids, and other small biological molecules, owing to their unique properties such as high chemical stability, good biocompatibility, and ability to facilitate electron transfer between electrodes and biomolecules [30]. Besides, AuNPs can form links with several compounds through Au-S or Au-NH chemical bonds or electrostatic interactions through negative charges. Here, we chose one 27-base sequence in the promoter region (exon 8) of the *Homo sapiens p53* gene for the target DNA, which has been proved to be a tumor-suppressor gene. The thiolated single-stranded DNA S1 probe was self-assembled onto the surface of AuNPs modified glassy carbon electrode (GCE) via Au−S bonding, followed by hybridization with target DNA S2 to form double-stranded DNA containing a specific recognition sequence of 5′-CCGG-3′ for both M.SssI MTase and resistant endonuclease *Hpa*II. It is known that the M.SssI MTase can transfer the methyl to the C-5 position of cytosine of the double-stranded DNA from SAM and the *Hpa*II can identify the duplex symmetrical sequence of 5′-CCGG-3′ and catalyze the digestion of double-stranded DNA between the un-methylated cytosine. The un-methylated double-stranded DNA could be specifically recognized and cleaved by *Hpa*II and the electrochemical signals of MB would decrease. However, the methylated double-stranded DNA could not be cleaved by HpaII because the recognition site of the resistant endonuclease was blocked. On the basis of them, we realized the detection of DNA methylation and assay of M. SssI MTase activity. Moreover, because the aberrant DNA methylation is closely related to many cancers, the screening of DNA MTase inhibitors is very important in the treatment of cancers. In this work, we also used the method to screen the inhibitors of M.SssI MTase, which may be helpful for the discovery of relevant anticancer drugs.



S1: 5′-SH-$(CH_2)_6$-CTC TGT GCG CCG GTC TCT CCC AGG ACA-3′
S2: 5′-TGT CCT GGG AGA GAC CGG CGC ACA GAG-3′
S3: 5′-TGT CCT GGG AGA GAC C*T*G CGC ACA GAG-3′

Scheme 1. Schematic illustration of the electrochemical assay for detection of DNA methylation and assay of DNA methyltransferase activity.