Biosensors and Bioelectronics 99 (2018) 201–208

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Highly efficient electrochemical sensing platform for sensitive detection DNA methylation, and methyltransferase activity based on Ag NPs decorated carbon nanocubes

Fenglei Gao[a,d], Taotao Fan[a], Shanshan Ou[a], Jing Wu[a], Xing Zhang[c], Jianjun Luo[c], Na Li[a], Yao Yao[a], Yingfeng Mou[d], Xianjiu Liao[b,*], Deqin Geng[c,d,**]

[a] Jiangsu Key Laboratory of New Drug Research and Clinical Pharmacy, Department of Pharmaceutical Analysis, School of Pharmacy, Xuzhou Medical University, 221004 Xuzhou, China
[b] School of Pharmacy, Youjiang Medical University for Nationalities, 533000 Baise, China
[c] The Graduate School, Xuzhou Medical University, 221004 Xuzhou, China
[d] Department of Neurology, Affiliated Hospital of Xuzhou Medical University, Xuzhou 221002, China

ARTICLE INFO

Keywords:
DNA methylation
Electrochemical
Carbon nanocubes
Inhibitor

ABSTRACT

In this paper, we reported a sensitive and selective electrochemical method for quantify DNA methylation, analyzing DNA MTase activity and screening of MTase inhibitor based on silver nanoparticles (Ag NPs) decorated carbon nanocubes (CNCs) as signal tag. The Ag NPs/CNCs was prepared by in situ growth of nanosilver on carboxylated CNCs and used as a tracing tag to label antibody. The sensor was prepared by immobilizing the double DNA helix structure on the surface of gold electrode. When DNA MTase was introduced, the probe was methylated. Successively, anti-5-methylcytosine antibody labeled Ag NPs/CNCs was specifically conjugated on the CpG methylation site. The electrochemical stripping signal of the Ag NPs was used to monitor the activity of MTase. The electrochemical signal has a linear relationship with M.SssI activities ranging from 0.05 to 120 U/mL with a detection limit of 0.03 U/mL. In addition, we also demonstrated the method could be used for rapid evaluation and screening of the inhibitors of MTase. The newly designed strategy avoid the requirement of deoxygenation for electrochemical assay, and thus provide a promising potential in clinical application.

1. Introduction

DNA methylation is an essential part of epigenetic modification, which influences the heritable state of gene expression (Esteller et al., 2001; Goren et al., 2003). DNA methylation in mammals occurs at cytosines in CpG dinucleotides by the addition of a methyl group to the C5-position of cytosines (Cao et al., 2012; Bi et al., 2013; Li et al., 2015). However, aberrant DNA methylation in these regions can result in transcriptional silence of tumor suppressor genes and be regarded as a hallmark of various diseases and cancers (Feng et al., 2008; Duan et al., 2010; Kurita et al., 2012). DNA methylation was regulated by DNA MTase in the presence of S-adenosylmethionine (SAM) which catalyzed cytosine or adenine in DNA sequences with a methyl group. The abnormity of DNA MTase activity would affect the level of DNA methylation, thus the investigation of DNA methylation and assay of DNA MTase activity have catalyzed considerable developments in the fields of genomics (Wu et al., 2013; Su et al., 2015; Zhang et al., 2016).

There are currently three main techniques for profiling DNA methylation and assay of MTase activity, chemical transformation, enzymatic cleavage, and affinity capture using methylated DNA binding proteins. These methods are usually time-consuming, labor-intensive and require specific reagents. Moreover, they do not provide accurate analysis of the degree of DNA methylation. Up to now, several new methods have been developed for the determination of DNA methylation and assay of MTases activity, such as methylation-specific polymerase chain reaction (Dai et al., 2012), colorimetry (Liu et al., 2009; Ge et al., 2012; Zhao et al., 2014), direct microarray-based assay (Wu et al., 2008), fluorescence assay (Zhang et al., 2014; Wang et al., 2014; Cao et al., 2014; Ma et al., 2015; Wen et al., 2016), SERS (Hu et al., 2011), HPLC (Chango et al., 2009), and HPLC-MS (Quinlivan et al., 2008; Lopez Torres et al., 2011) methods. Although these methods possess good specificity, they often suffer from poor sensitivity and low precision.

* Corresponding author at: School of Pharmacy, Youjiang Medical University for Nationalities, 533000 Baise, China.
** Corresponding author at: The Graduate School, Xuzhou Medical University, 221004 Xuzhou, China.
E-mail addresses: lxj2006910@163.com (X. Liao), gengdeqin@126.com (D. Geng).

http://dx.doi.org/10.1016/j.bios.2017.07.063
Received 22 April 2017; Received in revised form 25 July 2017; Accepted 25 July 2017
Available online 26 July 2017

Electrochemical methods have attracted more attention for the DNA methylation assay in the last three years because of the cheap instruments, simple operation, easy separation, high sensitivity and selectivity (Liu et al., 2011; Li et al., 2012; Xu et al., 2013). Various electrochemical biosensors based on labeling technologies with enzymes (Wang et al., 2012), electroactive substances (Wei et al., 2014), and nanoparticles (NPs) (Jing et al., 2014) have been developed to amplify the signal. Metal nanoparticles play an increasing role in biosensing due to their unique optical, electronic, and catalytic properties for translating of the biorecognition events to an electrochemical response. Compared with the enzyme tags, metal nanoparticles have shown great promise for sensitive bioelectrochemical tracing through stripping voltammetric analysis for the tagged metal nanoparticles in the dissolved oxygen interference-free potential range. Ag NPs possess good biocompatibility and have been extensively applied in biossays. Ag NPs can be directly detected using an electrochemical-stripping detection due to their lower oxidation potential and facile stripping conditions (Li et al., 2010; Song et al., 2014). However, few works using independent Ag NPs as the tag have been reported due to the limited stability of Ag NPs in saline buffer. Carbon materials, such as graphene and carbon nanotubes (Lai et al., 2011; Qu et al., 2011), have been reported to process favorable features catalyzing the silver deposition. Recently, carbon nanocubes as a kind of three-dimensional carbonaceous have gained great interest for various applications by virtue of its excellent properties, such as large functional surface area, high ratio of surface active groups to volume, biocompatible microenvironment, high electrical conductivity and many chemically active sites (Wang et al., 2010; Li et al., 2011; Tan et al., 2013). Thus this work combined the advantages of Ag NPs and CNCs to design synergistic Ag NPs/CNCs by in situ growth of nanosilver on CNCs. It has been reported that anti-5-methylcytosine antibody can specific recognition with methylated cytosine in both single-stranded and double-stranded DNA (Wang et al., 2012). On the basis of this, this work reported an electrochemical immune approach for the assay of MTase activity and the detection of DNA methylation of specific CpG sites. As illustrated in Scheme 1, CNCs were used as a "carrier" to load the Ag NPs tracers. In turn, thiolated DNA was self-assembled onto a gold electrode via Au–S bonding, followed by hybridization with DNA S2 to form double-stranded DNA containing a specific recognition sequence for M. SssI MTase. In the absence of M. SssI MTase, the reaction of DNA methylation had not been initiated. In the presence of M. SssI MTase, it could recognize the site-specific palindromic sequence 5-CCGG-3. The anti-5-methylcytosine antibody labeled Ag NPs/CNCs was specifically conjugated on the CpG methylation site. Therefore, M. SssI MTase activity and DNA methylation could be sensitively detected through the silver strip signals, which could be attributed to the powerful amplification effect of the Ag NPs/CNCs unit. Based on the novel Ag NPs/CNCs electrochemical sensing platform, the proposed sensor showed good sensitivity with wide linear range, providing a promising potential in future trace protein assay.

2. Materials and methods

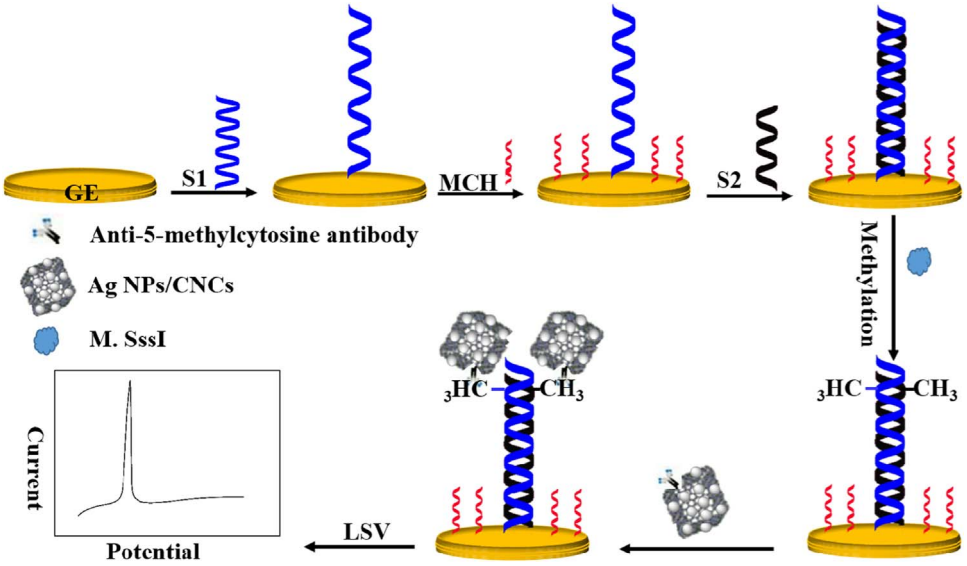
2.1. Reagents and materials

6-Mercapto-1-hexanol (MCH) was purchased from Alfa Aesar (Lancashire, England). Tris(hydroxymethyl)aminomethane (Tris), tri(2-carboxyethyl) phosphine hydrochloride (TCEP), $AgNO_3$, disodium ethylenediaminetetraacetic acid (EDTA) were purchased from Aladdin (Shanghai, China). Anti-5-methylcytosine antibody was purchased from Calbiochem (Merck KGaA, Darmstadt, GER). 10 μg/mL anti-5-methylcytosine antibody was prepared with 0.1 M PBS (pH 7.4). 5-aza-2-deoxycytidine was purchased from Sigma-Aldrich (St. Louis, USA). S-Adenosylmethionine (SAM) and M. SssI MTase is supplied by New England BioLabs (Ipswich, MA). The methyltransferase M. SssI MTase is stored at −20 °C in a buffer containing 10 mM Tris–HCl (pH 7.4), 50 mM NaCl, 1 mM dithiothreitol, 10 mM $MgCl_2$. All oligonucleotide sequences were synthesized by Shanghai Sangon Biotechnology Co. (Shanghai, China) and used without further purification. The base sequences of oligonucleotides were as following:

DNAS1:5′-SH-$(CH_2)_6$-GTACATCGTGTTCGCGACGCGCTACAGTCT CC GGAC-3′;
DNA S2: 5′-GTCCGGAGACTGTAGCGCGTCGCGAACACGATGTAC-3′;

2.2. Apparatus

Linear sweep stripping voltammetric (LSV) measurements were performed using a CHI 660B electrochemical workstation (CHI, Shanghai, China) at room temperature using a conventional three-electrode system with a modified GE as the working electrode, a platinum wire as the auxiliary, and a saturated calomel electrode as the reference. LSV from −0.15 to 0.25 V at 50 mV s^{-1} was performed in a



Scheme 1. Schematic representation of the developed method for detection of DNA methylation and assay of M. SssI MTase activity based on the electrochemical signal amplification of Ag NPs/CNCs.