Journal of Electroanalytical Chemistry 773 (2016) 63–68

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

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

Methylation-specific electrochemical biosensing strategy for highly sensitive and quantitative analysis of promoter methylation of tumor-suppressor gene in real sample

Shenglin Liu [a,b], Xuejun Zhang [a], Kebin Zhao [c,*]

[a] Department of Immunology, Tianjin Medical University, Tianjin 300070, China
[b] Department of Laboratory Medicine, Xiqing Hospital of Tianjin, Tianjin 300380, China.
[c] Department of Laboratory Medicine, The First Hospital of Shanxi Medical University, Taiyuan 030001, China

ARTICLE INFO

Article history:
Received 14 December 2015
Received in revised form 27 February 2016
Accepted 2 March 2016
Available online 18 March 2016

Keywords:
Electrochemical biosensor
DNA methylation
Tumor suppressor gene
High sensitivity
Practicability

ABSTRACT

A handy and practical methylation-specific electrochemical biosensing strategy was developed by integrating bisulfite conversion and PCR amplification with high efficient electrochemical biosensor. The dual signal amplification of PCR and enzyme-catalyzed electrochemical sensing resulted in higher sensitivity of the proposed method. The primers for PCR and capture probes for amplicons binding were specifically designed to distinguish different CpG islands in the promoter regions of target gene, which further enhanced the specificity. The designed method showed very high sensitivity and specificity for DNA methylation analysis and was able to respond to about 8 copies of methylated promotor of secreted frizzled-related protein 2 (SFRP2) gene in 10,000 times of unmethylated genomic DNA. Moreover, the established biosensor was successfully verified for quantitative detection of SFRP2 promotor methylation in breast primary tumor tissues and subtlemonitor of SFRP2 promotor methylation status changes in real sample with the demethylating agent treatment. This proposed strategy presented a simple and pragmatic platform toward high sensitive and quantitative detection promoter methylation of tumor-suppressor gene for early diagnosis and prognostic assessment of cancer, as well as monitoring response to therapeutic agents.

1. Introduction

DNA methylation is the best-known epigenetic marker, which refers to that cytosine is modified with a methyl group at the 5′-position by the catalysis of a group of DNA methyltransferase enzymes after DNA replication. DNA methylation plays a critical role in many biological events, such as control of tissue-specific genes and germ-line genes, genomic imprinting, X-chromosome inactivation and prevention of chromosomal instability [1–3]. Aberrant DNA methylation is frequently observed in tumor cells with global hypomethylation and hypermethylation of the CpG islands in the promoter regions of tumor-suppressor genes [4,5]. Furthermore, hypermethylation of the CpG islands is an early event in the development of cancer [6]. Thus, quantitative analysis of DNA methylation of the CpG islands in the promoter regions of tumor-suppressor genes can be serve as potential tumor biomarker for early diagnosis of cancer, monitoring tumor behavior, as well as measuring response of tumors to targeted therapy [7–9].

So far, many techniques have been applied to profile the methylation status of target genes. Generally, these DNA methylation analysis techniques mainly rely on either methylation-sensitive restriction enzyme (MSRE) digestion or sodium bisulfite conversion to distinguish methylated cytosine from unmethylated cytosine [10,11]. MSRE-based methods are fast and simple, which use MSRE to cleave DNA at specific unmethylated cytosine residues and leave the methylated DNA intact, are fast and simple [12,13]. But the dependence upon methylation-sensitive restriction sites limits their wide applications due to methylation of CpG dinucleotides that may vary within one CpG island [13,14]. Alternatively, the most widely applied methods for analysis of DNA methylation rely on sodium bisulfite to treat genomic DNA for converting cytosines, but not 5-methylcytosines, to uracil. In traditional methylation-specific PCR (MSP), the sodium bisulfite converted genomic DNA can be amplified with specific primers and the products are identified using gel electrophoresis [11]. However, this standard MSP approach offers only qualitative analysis result and has relative low sensitivity [15]. Real-time methylation-specific PCR provides a quantitative, high-throughput and real-time resolution [16,17], but double-labeled probes and quantitative PCR apparatus make it too expensive and difficult to popularize [18]. Thus, the simple, inexpensive method for high sensitive and quantitative detection of DNA methylation is still an urgent demand.

In recent years, extensive efforts have been devoted to develop new biosensing strategies for high sensitive and specific analysis of DNA methylation, including fluorescence [19,20], surface plasmon resonance [21,22], electrochemistry [23], electrogenerated chemiluminescence [24] and colorimetry [25]. These biosensing strategies are mostly

* Corresponding author.
E-mail address: sxzhaokebin@126.com (K. Zhao).

http://dx.doi.org/10.1016/j.jelechem.2016.03.001
1572-6657/

based on MSRE, which really provide useful tools for analyzing demethylase activity and screening demethylase inhibited drug. However, the inherent defect of restriction sites limits their application for comprehensive profiling methylation status of the CpG islands of target genes. In addition, the methyl-CpG binding domain (MBD) protein and anti 5-methylcytidine antibody were employed as recognition elements to build various biosensor for rapid analysis cytidine methylation [20,22,26]. But these biosensors lack the nucleic acid amplification like PCR or isothermal DNA amplification, constraining the sensitivity and practicability of these biosensors for the analysis of real specimens containing low amounts of methylated DNA. Moreover, some nanomaterials-based biosensors have been designed to analyze the MSP product for improving the quantitative capability and analytical sensitivity of MSP [18,27]. However, the variability of the nanomaterials and their bio-functionalization often affects the reproducibility and quantification, especially for the real samples [28].

Herein, aiming at further improving the simplicity, practicality and sensitivity of biosensor-based DNA methylation detection, a handy methylation-specific electrochemical biosensing strategy was developed by integrating bisulfite conversion and PCR amplification with high efficient electrochemical biosensor (Fig. 1). The dual signal amplification of PCR and enzyme-catalyzed electrochemical sensing resulted in higher sensitivity of the proposed method. The primers for PCR and capture probes for amplicons binding were specifically designed to distinguish different CpG islands in the promoter regions of target gene, which further enhanced the specificity. Secreted frizzled-related protein 2 (SFRP2) gene, an important tumor suppressor gene, can down-regulate Wnt signaling and antagonize tumorigenesis. The promoter hypermethylation of SFRP2 gene leads to abrogation of SFRP2 expression, activation of Wnt signaling, resulting in tumorigenesis [29]. Promoter hypermethylation of the SFRP2 gene is proved a high-frequent alteration in human cancers, and is qualified as a potential candidate biomarker for early detection and invasive prediction of cancer [29–31]. Therefore, this work used SFRP2 gene as a model to verify the practicability of the designed strategy. The proposed methylation-specific electrochemical biosensing protocol showed very high sensitivity and selectivity and was successfully applied to detection methylation status of SFRP2 promoter in breast primary tumor tissues and demethylation drug treated breast cancer cell lines. Thus, the designed electrochemical biosensing strategy presented a simple, pragmatic and inexpensive platform toward high sensitive and quantitative detection of promoter methylation of tumor-suppressor gene for early diagnosis of cancer.

2. Experimental

2.1. Reagents

6-Mercapto-1-hexanol (MCH), Streptavidin-alkaline phosphatase (ST-AP), α-naphthyl phosphate (α-NP), bovine serum albumin (BSA), salmon sperm DNA and 5-aza-2′-deoxycytidine (Aza) were purchased from Sigma-Aldrich (USA). Trichostatin A (TSA) were purchased from Cayman Chemical Co. (USA). AmpliTaq DNA Polymerase was purchased from ABI (USA). The oligonucleotides with the sequences shown in Table 1 were obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (China). All other reagents were of analytical grade. All aqueous solutions were prepared using ultra-pure water (≥18 MΩ, Milli-Q, Millipore).

2.2. Apparatus

All electrochemical measurements were performed on a CHI660D electrochemical analyzer (CHI Co., TX) with a conventional three-electrode system composed of platinum wire as auxiliary, Ag/AgCl

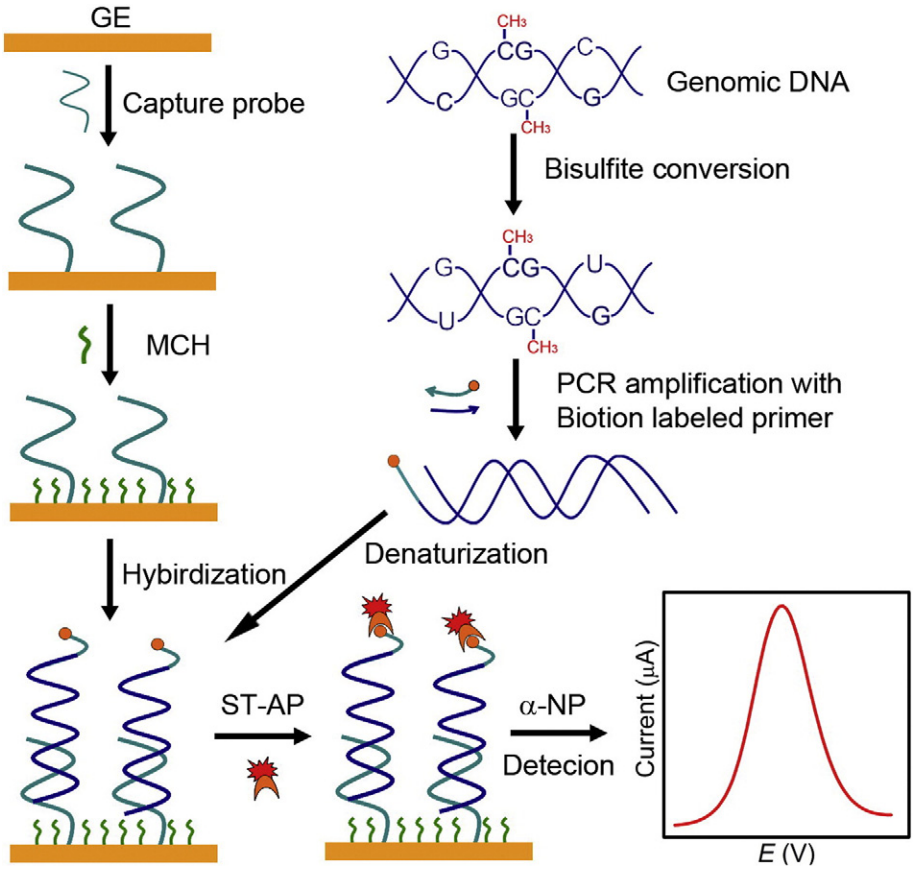

Fig. 1. Schematic presence of methylation-specific electrochemical biosensing strategy for DNA methylation detection.