Biosensors and Bioelectronics 60 (2014) 35–44

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

DNA methylation detection by a novel fluorimetric nanobiosensor for early cancer diagnosis

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ARTICLE INFO

Article history:
Received 10 February 2014
Received in revised form
12 March 2014
Accepted 15 March 2014
Available online 15 April 2014

Keywords:
Nanobiosensor
Fe@Au nanoparticle
DNA methylation
Dipyridamole
Fluorescence

ABSTRACT

A very sensitive and convenient fluorescence nanobiosensor for rapid detection of DNA methylation based on Fe_3O_4/Au core/shell nanoparticles has been developed. Specific site of CpG islands of adenomatous polyposis coli (APC), a well studied tumor suppressor gene, was used as the detection target DNA sequence. The characteristics of nanoparticles were determined by scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy dispersive spectroscopy (EDS), UV–visible spectroscopy and X-ray diffraction (XRD) spectroscopy. Fe@Au nanoparticles functionalized by bounding of single stranded DNA (ssDNA) probe through sulfhydryl group at the 5′ phosphate end. Then unmethylated and methylated complementary target ssDNA were hybridized with the immobilized ssDNA probe. Dipyridamole, a pharmaceutical agent used for the first time as a fluorescence probe which significantly interacted with hybridized unmethylated and methylated DNA. Upon the addition of the target unmethylated and methylated ssDNA, the fluorescence intensity increased in linear range by concentration of unmethylated ssDNA from 1.6×10^{-15} to 6.6×10^{-13} M with detection limit of 1.2×10^{-16} M and on the other hand, fluorescence intensity declined linearly with concentration of 3.2×10^{-15}–8.0×10^{-13} M methylated DNA and detection limit was 3.1×10^{-16} M. We have also shown that nanobiosensor could distinguish ratio of methylation in series of partially methylated DNA targets with identical sequences. A density functional theory (DFT) calculation was also performed to investigate the interaction between Dipyridamole with unmethylated and methylated cytosine. Finally real sample analysis suggested that nanobiosensor could have practical application for methylation detection in human plasma sample.

1. Introduction

Epigenetics is defined as those heritable changes in gene expression that are not due to any alteration in DNA sequence (Holliday, 1987). It mainly includes DNA methylation, RNA associated silencing and histone posttranslational modification (Kouzarides, 2007). Among them, DNA methylation is the most common DNA modification and best known epigenetic marker that is almost found on the 5′ position of the pyrimidine ring of cytosine base in the CpG dinucleotides (Bird, 2002). During the evolution, the dinucleotide CpG has been eliminated from the genome except from small regions of DNA, called CpG islands (Herman and Baylin, 2003). Most CpG islands are 2 kb away from promoter and in active genes are usually unmethylated (Ndlovu et al., 2011). A vast majority of these unmethylated CpG islands are found in the upstream promoter regions of approximately 50% of genes, but in others they are located downstream of the transcription start point (Yan et al., 2006). After completed development, DNA methylation patterns are frozen but unprogrammed changes in DNA methylation patterns can lead to cancer by an altered expression of multiple genes. Hypermethylation, which represses transcription of the promoter regions of tumor suppressor genes leading to gene silencing, has been most extensively studied (Kristensen et al., 2009).

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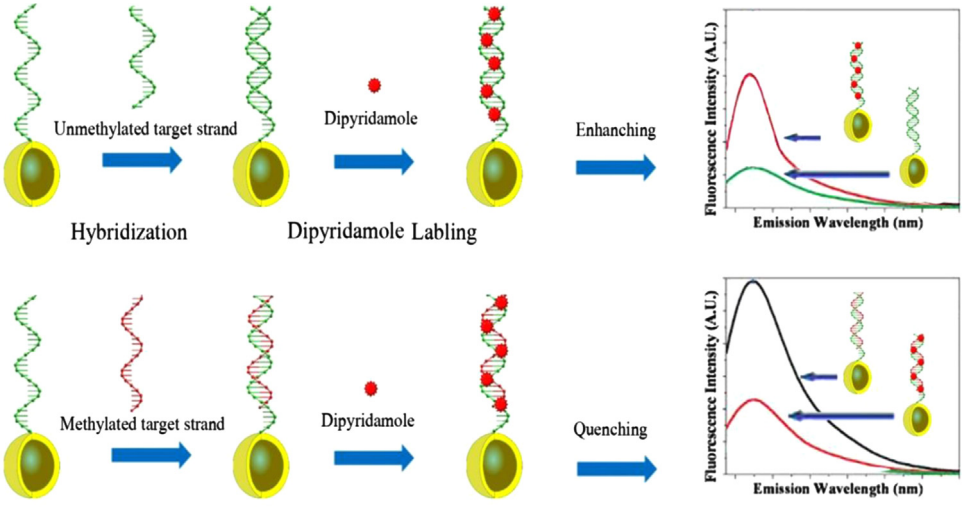
http://dx.doi.org/10.1016/j.bios.2014.03.033
0956-5663/

With respect to molecular function, DNA methylation can inhibit gene expression either by the binding of transcription factors or through the recruitment of methyl binding domain (MBD) proteins and other chromatin factors, which will induce a closed state of the chromatin (heterochromatin) and abolish transcription (Klose and Bird, 2006; Bogdanovic and Veenstra, 2009). It also has a pivotal role in proper development and has been found implicated in X chromosome inactivation (Escamilla-Del-Arenal et al., 2011), silencing of repetitive elements, genomic imprinting (Abramowitz and Bartolomei, 2012) as well as disease occurrence particularly tumorigenesis (Hatziapostolou and Iliopoulos, 2011). So DNA methylation can serve as a reliable biomarker for early cancer diagnosis.

Adenomatous polyposis coli (APC) is a tumor suppressor gene, and its gene product indirectly regulates transcription of a number of genes that are crucial for cell proliferation (Fearnhead et al., 2001). Hypermethylation of this gene has been confirmed in many cancers (Sheikhnejad et al., 2013; Kawasaki et al., 2013) so a specific sequence of APC gene was selected as the target in the present study.

Analysis of methylated DNA in the blood or other body fluids could be reliable and useful approach for cancer screening (Ma et al., 2013). The limited amounts of DNA present in body fluids, plasma and serum require very sensitive assays. Regarding to this sensitivity, multiple DNA methylation assays have been developed, which are based on methods such as sodium bisulfite (NaBi) based assays that is modification of the DNA that converts unmethylated cytosines to uracil to distinguish methylated from unmethylated DNA (Clark et al., 1994). But these approaches have been subjected to many limitations which include material handling and most reliable detection. Bisulfite conversion causes degradation of $>90\%$ of the input DNA (Clark et al., 2006) and factors such as contamination of proteins and DNA quality contribute to the unsatisfactory conversion efficiency (Warnecke et al., 2002).

Recently some new methods such as DNA methylation assay based on single base extension reaction and surface enhanced Raman spectroscopy (SERS) (Hu and Zhang, 2012), surface plasmon resonance (SPR) (Yu et al., 2010), methylation-specific microarray (Wu et al., 2008) and methylation-specific fluorescence resonant energy transfer (MS-FRET) (Bailey et al., 2009) have been developed. Also in another study, Cipriany et al. (2012) integrated nanofluidics and sorting technology to identify methyl binding domain protein-1 (MBD1) bounded to fluorescence labeled DNA in real time analysis. But it should be noted that in all above studies the requirement of professional design and operations still restricts their application. So it is necessary to explore a simple method for DNA methylation detection based on fluorescence technology due to its high sensitivity, better specificity and also easy operation. Here we present for the first time the fluorimetric detection assay based on Dipyridamole, a novel fluorescence DNA probe. Dipyridamole (2,6-bis (diethanolamino)-4,8-dipiperidinopyrimido-(5,4-d-pyrimidine)) is a well known and widespread pharmaceutical agent used for the treatment of angina pectoris and myocardial infarction (Fitzgerald, 1987; Steimberg, 1992). It is characterized by an aromatic central ring system which is responsible for intense π–π* transition absorption bands in the 400 and 280 nm region (Borissevitch and Tabak, 1992; Martín et al., 1988, 2002; Petersson et al. 1998), and for an intense fluorescence band centered at 500 nm (Borissevitch et al., 1995). The study of interaction of drugs with DNA is very exciting and significant not only in understanding the mechanism of interaction, but also for the design of new drugs (Sirajuddin et al., 2013). So far there is little knowledge about the exact mode of interaction mechanism between drug molecules and DNA and also there is no report about utilization of the potent characteristic of pharmaceutical drug for detection methods. Using nanoparticles have the potential to improve the biosensors utilities and may result in cheaper, faster and easy to use analytical tools (Chauhan et al., 2012). Furthermore, nanoscale biosensors may be more portable and scalable for point of care sample analysis and real time diagnosis. Core–shell magnetic nanoparticles comprise a magnetic core (e.g. iron oxide, cobalt, etc.) and a shell can provide not only a protective layer to the nanoparticles but also a platform for the surface functionalization of the nanoparticles (Lin et al., 2001). Due to the magnetic properties of these nanoparticles, they can readily be isolated from sample solutions by the application of an external magnetic field. The Au layer can be expected to protect the magnetite from oxidation in harsh environments, and another feature is that the Au surface can be readily functionalized through well developed Au-Thiol group bindings. To explore the magnetic properties, the formation of a gold shell with a controllable size formation allows better stability and tunability for the construction of nanobiosensors (Wang et al., 2005). In this study Fe@Au nanoparticles have been used for immobilizing of ssDNA probe and magnetically separation of captured ssDNA targets and Dipyridamole used as a fluorescence probe that binds and interacts with double stranded DNA which distinguishs between methylated and unmethylated DNA sequences through different fluorescence behavior. An overview of the detection mechanism is illustrated in Scheme 1.



Scheme 1. Schematic representation of the assay for DNA methylation detection.