Biosensors and Bioelectronics 54 (2014) 285–291

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Carbon nanotube signal amplification for ultrasensitive fluorescence polarization detection of DNA methyltransferase activity and inhibition

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ARTICLE INFO

Article history:
Received 25 July 2013
Received in revised form
2 October 2013
Accepted 21 October 2013
Available online 13 November 2013

Keywords:
Multiwalled carbon nanotubes
Fluorescence polarization
Nanosensors
DNA methyltransferase
Inhibition

ABSTRACT

A versatile sensing platform based on multiwalled carbon nanotube (MWCNT) signal amplification and fluorescence polarization (FP) is developed for the simple and ultrasensitive monitoring of DNA methyltransferase (MTase) activity and inhibition in homogeneous solution. This method uses a dye-labeled DNA probe that possess a doubled-stranded DNA (dsDNA) part for Mtase and its corresponding restriction endonuclease recognition, and a single-stranded DNA part for binding MWCNTs. In the absence of MTase, the dye-labeled DNA is cleaved by restriction endonuclease, and releases very short DNA carrying the dye that cannot bind to MWCNTs, which has relatively small FP value. However, in the presence of MTase, the specific recognition sequence in the dye-labeled DNA probe is methylated and not cleaved by restriction endonuclease. Thus, the dye-labeled methylated DNA product is adsorbed onto MWCNTs via strong π–π stacking interactions, which leads to a significant increase in the FP value due to the enlargement of the molecular volume of the dye-labeled methylated DNA/MWCNTs complex. This provides the basic of a quantitative measurement of MTase activity. By using the MWCNT signal amplification approach, the detection sensitivity can be significantly improved by two orders of magnitude over the previously reported methods. Moreover, this method also has high specificity and a wide dynamic range of over five orders of magnitude. Additionally, the suitability of this sensing platform for MTase inhibitor screening has also been demonstrated. This approach may serve as a general detection platform for sensitive assay of a variety of DNA MTases and screening potential drugs.

1. Introduction

DNA methyltransferase (MTase)-mediated DNA methylation is an important epigenetic event that plays a pivotal role in DNA repair, gene transcription, and embryogenesis (Choy et al., 2010). Moreover, the aberrant methylation of CpG islands in promoter regions of genes is a new generation of cancer biomarkers, and can be regarded as a hallmark of various diseases (Jaenisch and Bird, 2003; Jeltsch et al., 2007). Additionally, DNA MTase is also a novel family of pharmacological targets for the treatment of tumors (Esteller et al., 2001; Szyf, 2005). Thus, evaluations of DNA MTase activity and inhibition are essential for clinical diagnostics and drug development. Traditional assay methods such as radioactive labeling, gel electrophoresis and high performance liquid chromatography (Som and Friedman, 1991; Rebeck and Samson, 1991; Reenila et al., 1995) have been established for DNA MTase detection. However, these methods are time-intensive, DNA-consuming, laborious, not sensitive, or require isotope labeling. Many of these limitations are now being addressed by the development of some new methods (Li et al., 2012, 2013; Liu et al., 2010; Tian et al., 2012). For example, fluorescence assays based on hairpin fluorescence molecular beacon based DNA probe, cationic conjugated polymer/DNA complexes and graphene oxide-mediated DNA probe have been developed for assaying DNA MTases (Li et al., 2007; Feng et al., 2007). Colorimetric assays based on gold nanoparticle (AuNP) aggregation or horseradish peroxidase-mimicking DNAzyme were reported for detection of DNA MTase (Song et al., 2009; Li et al., 2010). In addition, electrochemical biosensors based on the use of ferrocene, alkaline phosphatase or AuNPs as labels have also been developed for assay of DNA MTase activity (He et al., 2011; Wang et al., 2010; Liu et al., 2011; Wu et al., 2012). Albeit substantial progress was accomplished, a method that is simpler and more sensitive still is necessitated.

Carbon nanotubes (CNTs) attracts growing interest as a new, water-soluble material for biological studies due to their unique structural, mechanical and optoelectronic properties (Wang et al., 2013; Chen et al., 2011). Particularly interesting is the interaction of nucleic acids with CNTs. It is found that single-stranded nucleic

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http://dx.doi.org/10.1016/j.bios.2013.10.065

acids adsorb strongly on CNTs, while duplex DNAs cannot bind to MWCNTs stably (Tang et al., 2006). This property has been employed to elaborately design sensors for various molecular targets in homogeneous solution (Zhang et al., 2010a; Wu et al., 2008). For example, the desorption of a fluorophore-labeled single-stranded DNA by the complementary nucleic acid was used for rapid and sensitive fluorescence detection of nucleic acids (Liu et al., 2009; Yang et al., 2008; Zhu et al., 2008). Similarly, the desorption of fluorophore-labeled nucleic acids from CNTs through the generation of aptamer-substrate complexes was implemented to develop CNT-based sensor systems for fluorescent detection of biomolecules (Zhen et al., 2010; Zhang et al., 2011a). In addition, the release of fluorophore-labeled metal-specific oligonucleotides from CNTs through the formation of metal ion-mediated duplex DNA structures was also used for the fluorescence detection of metal ions (Zhao et al., 2010; Zhang et al., 2010b). Although these techniques can be quite powerful, greater sensitivity and specificity are often required, particularly when working with limited amounts of sample material or when target concentration is extremely low.

Fluorescence polarization (FP) is a simple signaling approach that provides a quantitative measure for the rotational motion of a fluorescently labeled molecule. The FP value *P* is sensitive to changes in the rotational motion of fluorescently labeled molecules, which depends on the size of molecule. If a fluorescence molecule is free in solution, it rotates at a rate commensurate with its size and hence will have a relatively small P value. However, when the fluorescence molecule binds with another substance to form a complex, its rotational rate decreases and the *P* value will increase; the degree of variation depends on the strength of the binding interaction and the size of the formed complex (Zhang et al., 2011b; Deng et al., 2007). This technique has found widespread applications in monitoring the binding events of biomolecules and asssaying of various biomolecules or cells (Deng et al., 2010; Ruta et al., 2009; Zhang et al., 2011c, 2012; Perrier et al., 2010). Recently, to improve the detection sensitivity, several amplifying strategies that employ proteins (Cui et al., 2012; Zhu et al., 2012), AuNPs (Yin et al., 2010; Ye and Yin, 2008; Huang et al., 2011), silica nanoparticles (Huang et al., 2012), and graphene oxide (Liu et al., 2013; Yu et al., 2013) as FP enhancers for detection of small molecules and proteins have been proposed. Nonetheless, FP-based sensing platforms that implement CNTs as a signal amplifier are at present unknown.

In the present work, we report a novel amplified sensing platform based on multiwalled carbon nanotube (MWCNT) signal amplification and fluorescence polarization (FP) for highly sensitive and selective detection of DNA MTase activity and inhibition. To the best of our knowledge, this is the first example of using CNTs as a signal amplifier for FP assay of biomolecules. Compared with traditional methods for DNA MTase assays, this proposed protocol does not require separation and troublesome procedures, which is very simple and fast. Most importantly, the introduction of MWCNTs causes a significant amplification of the detection signal, which substantially improves the detection sensitivity by two orders of magnitude over the previously reported methods. Moreover, the application of this MWCNT-based FP sensing platform for DNA MTase inhibitor screening has also been demonstrated.

2. Experimental

2.1. Materials and reagents

All oligonucleotides were purchased from the Sangon Biotech Co. (Shanghai, China) and purified by HPLC. The sequences of the involved oligonucleotides were as follow: 5′-CGA TCC CGC TGC CGG GCC CCG CTG CCC TGT GCC GAA TTT TTT-3′ (DNA-1) and 5′-GGC CCG GCA GCG GGA TCG-FAM-3′ (cDNA-1) were used for Dam MTase detection; and 5′-GTG AAT TCC ATC CGA CCC CCG CTG TCA TGT GCC GAA TTT AAA -3′ (DNA-2) and 5'-GGG TCG GAT GGA ATT CAC-FAM-3′ (cDNA-2) were used for assaying EcoRI MTase. The Dam MTase, AluI MTase, EcoRI MTase, DpnII endonuclease, EcoRI endonuclease and S-adenosyl-L-methiolnine (SAM) were purchased from New England Biolabs (NEB, UK). Standardized human serum was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Multiwalled carbon nanotubes (MWCNTs) were purchased from Shenzhen Nanotech Port Co. Ltd. (Shenzhen, China), and oxidized and purified as described previously (Zhen et al., 2010). Other chemicals were of analytical grade. Water was purified by using a Milli-Q plus 185 equip from Millipore (Bedford, MA).

2.2. Apparatus

Fluorescence polarization (FP) measurements were carried out using an FL3-P-TCSPC system (Jobin Yvon, Inc., Edison, NJ, USA) with 300 μL cuvette. The FP of the sample solution was monitored by exciting the sample at 494 nm and measuring the emission at 520 nm (Fig. S1). And slits for both the excitation and the emission were set at 5 nm. HPLC analysis was performed by using an LC-10ATVP system equipped with RF-10AXL fluorescence detector (Shimadzu, Kyoto, Japan). The assays were carried out on a C18 column (250 × 4.6 mm i.d., 5 μm particle sizes, Elite, China). The HPLC assay used 494 nm and 520 nm as excitation and detection wavelengths, respectively.

2.3. DNA MTase activity assay

To prepare duplex DNA substrate of Dam MTase (dsDNA-1), 2.5 μL of 100 μM FAM-labeled DNA-1 was mixed with a 1.2-fold excess of cDNA-1 in pH 7.5 buffer containing 50 mM Tris–HCl and 50 mM NaCl. Then, the mixture was annealed by heating to 90 °C for 5 min and followed by slow cooling at room temperature for 1 h. For Dam MTase assay, reaction mixture was prepared by mixing Dam MTase enzyme stock with 45 nM annealed dsDNA-1 in 200 μL reaction buffer (pH 7.5 Tris–HCl buffer containing 50 mM Tris, 50 mM NaCl, 10 mM dithiothreitol, 0.3 mM SAM) and incubated at 37 °C for 1 h. Then, 50 μL of DpnII reaction buffer containing 50 units of DpnII was added and incubated for 30 min. Finally, 50 μL of MWCNTs solution were added to the above reaction mixture to give a final concentration of 60 μg/mL, and incubated for another 30 min. The obtained sample solution was used for FP measurements. FP was measured by using the L-format configuration and FluorEssence™ software with constant wavelength analysis to achieve a FP value. The G factor was initially set to zero, to let the system measure G automatically. The FP value was also calculated automatically by the instrument. The integration time was set to 3 s for the FP measurements. Over five FP measurements were taken each time, and they were then averaged for further data processing. In case of EcoRI MTase, duplex DNA substrate of EcoRI MTase (dsDNA-2) was annealed by mixing 2.5 μL of 100 μM FAM-labeled DNA-2 and a 1.2-fold excess of cDNA-2 in pH 7.5 buffer containing 50 mM Tris–HCl and 50 mM NaCl. Then, the mixture was annealed by heating to 90 °C for 5 min, followed by slow cooling at room temperature for 1 h. Other procedure for EcoRI MTase activity assay was similar to detection of EcoRI MTase activity mentioned above, except that Dam MTase, dsDNA-1 and DpnII endonuclease were replace of EcoRI MTase, and dsDNA-2, and EcoRI endonuclease, respectively.