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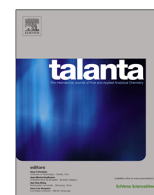

A fluorescence method for detection of DNA and DNA methylation based on graphene oxide and restriction endonuclease HpaII

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ABSTRACT

DNA methylation plays an important role in many biological events and is associated with various diseases. Most traditional methods for detection of DNA methylation are based on the complex and expensive bisulfite method. In this paper, we report a novel fluorescence method to detect DNA and DNA methylation based on graphene oxide (GO) and restriction endonuclease HpaII. The skillfully designed probe DNA labeled with 5-carboxyfluorescein (FAM) and optimized GO concentration keep the probe/target DNA still adsorbed on the GO. After the cleavage action of HpaII the labeled FAM is released from the GO surface and its fluorescence recovers, which could be used to detect DNA in the linear range of 50 pM–50 nM with a detection limit of 43 pM. DNA methylation induced by transmethylase (Mtase) or other chemical reagents prevents HpaII from recognizing and cleaving the specific site; as a result, fluorescence cannot recover. The fluorescence recovery efficiency is closely related to the DNA methylation level, which can be used to detect DNA methylation by comparing it with the fluorescence in the presence of intact target DNA. The method for detection of DNA and DNA methylation is simple, reliable and accurate.

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1. Introduction

Genomic DNA methylation is one of the most important epigenetic modifications in eukaryotes, which serves a wide variety of biological functions. DNA transmethylase (Mtase) can catalyze the transfer of a methyl group to DNA and all the known DNA methyltransferases use S-adenosylmethionine (SAM) as the methyl donor. In animals, most of the methylations occur at the 5′ position of the pyrimidine ring of the cytosine. The methylcytosine (mC) is mainly found in cytosine–guanine (CpG) dinucleotides. The presence of 5-mC in the promoter of specific genes alters the binding of transcriptional factors and other proteins to DNA and recruits methyl-DNA-binding proteins and histone deacetylases that compact the chromatin around the gene-transcription start site. Both mechanisms block transcription and cause gene silencing. Thus methylation of cytosine residues in genomic DNA plays a key role in the regulation of gene expression in many biological events and is closely associated with various diseases, especially cancer [1–3]. So, it is significant to develop a simple and sensitive method for the detection of DNA methylation.

The traditional method for detection of DNA methylation is based on bisulfite. Bisulfite modification converts unmethylated cytosine to uracil, while methylated cytosine cannot react. After denaturation and bisulfite modification, double-strand DNA is obtained by primer extension and the fragment of interest is amplified by PCR. There is an extensive range of methods based on the sodium bisulfite treatment for quantifying the methylation status of cytosines located in specific DNA regions [4,5]. The bisulfite-based methods are currently considered gold standard assay techniques because they are reliable, accurate, and can help understand the methylation status of each CpG in target DNA, while complex and expensive clone and sequencing procedures are needed in this method. A lot of new methods, including gel electrophoresis [6], real-time quantitative polymerase chain reaction [7], high-performance liquid chromatography (HPLC) [8,9] and gas chromatography/mass spectrometry (GC/MS) [10], are also developed. They are also complicated to operate, time consuming and expensive. Recently, alternative approaches such as electrochemical [11–13], electrogenerated chemiluminescence strategy [14,15], colorimetric [16–19] and fluorescence methods [20–25]

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have been developed to detect DNA methylation. Endonucleases can recognize and cleave specific short DNA sequences. Combined with methyltransferase they are usually used to detect DNA methylation [26–29]. GO has been reported as an excellent quenching material for the fluorescence dye due to fluorescence resonance energy transfer. The other superior property is that GO has a strong binding force with ssDNA strands due to π-stacking interaction between nucleobases and GO surface [30–33].

Herein, a graphene oxide (GO) based platform coupled with HpaII and Mtase M.SssI is studied to detect DNA methylation. First, GO was used to quench the probe DNA that was modified with 6-carboxyfluorescein (FAM) on its 5′ end. The probe DNA are skillfully designed to have 10 bases longer than that of target DNA in order to make their hybridism still adsorbed on the GO surface (Scheme 1, a). After the cleavage of specific site 5′-CCGG-3′ through HpaII, the labeled FAM is released to the solution and the fluorescence recovers (Scheme 1, b). The DNA detection limit of this method is 43 pM without signal amplification strategy. When the target DNA is methylated, the fluorescence cannot recover because HpaII digestion function is blocked by methylated cytosine base (Scheme 1, c). Therefore, the fluorescence intensity of FAM is closely related to the methylation level. Based on this, a sensitive fluorescence method for detection of DNA and DNA methylation is proposed. The method avoids bisulfite treatment of DNA and is simple, reliable and selective.

2. Experimental

2.1. Reagents and apparatus

The GO was synthesized by our group following the Hummers method [34]. Tris(hydroxymethyl)aminomethane (Tris) was purchased from Sigma-Aldrich. Hydrogen peroxide (30% in water) (H_2O_2) was purchased from Sinoreagent. S-adenosylmethionine (SAM), *Escherichia coli* CpG methyltransferase (M.SssI), and *E. coli* restriction endonuclease (HpaII) were obtained from New England BioLabs (NEB, UK). All these standard solutions were prepared and stored at under 4 °C. All other chemical reagents were of analytical reagent grade and used without further purification. Millipore Milli-Qwater (18 MΩ cm) was used throughout.

The DNA oligonucleotides were synthesized by Shanghai Sangon Biological Engineering Technology and Services Co., Ltd., China. 1 μM DNA stock solution was prepared by using 33 mM Tris–acetate buffer solution (pH 7.4) containing 66 mM NaAc and 10 mM $Mg(Ac)_2$. The sequences of oligonucleotides used in this work are shown in Table 1.

The buffers employed in this work were as follows: DNA hybridization buffer was 33 mM Tris–acetate (pH 7.4) containing 66 mM NaAc and 10 mM $Mg(Ac)_2$. The buffer for MTase work was 10 mM Tris–HCl (pH 7.9) containing 50 mM NaCl, 10 mM MgCl, and 1 mM DTT. The buffer for endonuclease HpaII digestion experiment was 33 mM Tris–acetate (pH 7.9) containing 10 mM $Mg(Ac)_2$, 66 mM KAc and 0.1 mg/mL BSA.

Safety note: dimethyl sulfoxide (DMSO) and acetaldehyde (CH_3CHO) are suspected human carcinogens and somewhat volatile. Gloves were worn; manipulations were done under a closed hood. All reactions were done in closed vessels.

A fluoromax 4 spectrofluorometer (Horiba, Japan) was used in the experiment.

2.2. Endonuclease digestion of probe/target DNA on GO surface

FAM-labeled probe DNA was mixed with target DNA (10 bases longer than probe DNA) in 33 mM pH 7.4 Tris–acetate buffer solution containing 66 mM NaAc and 10 mM $Mg(Ac)_2$. Then, the solution was heated to 90 °C for 5 min followed by slow cooling to room temperature to ensure complete hybridization. Then, HpaII was incubated with probe/target DNA in 33 mM pH 7.9 Tris–acetate buffer solution containing 10 mM $Mg(Ac)_2$, 66 mM NaAc and 0.1 mg/mL BSA at 37 °C. Finally, 25 μg/mL GO was added to the mixture and the fluorescence intensity of the FAM was monitored.

2.3. DNA cytosine methylation induced by M.SssI Mtase

The methylation was prepared by incubating M.SssI MTase with prepared double strands DNA in 1× MTase reaction buffer (10 mM pH 7.9 Tris–HCl containing 50 mM NaCl, 10 mM MgCl, 1 mM dithiothreitol and 160 μM SAM) at 37 °C.

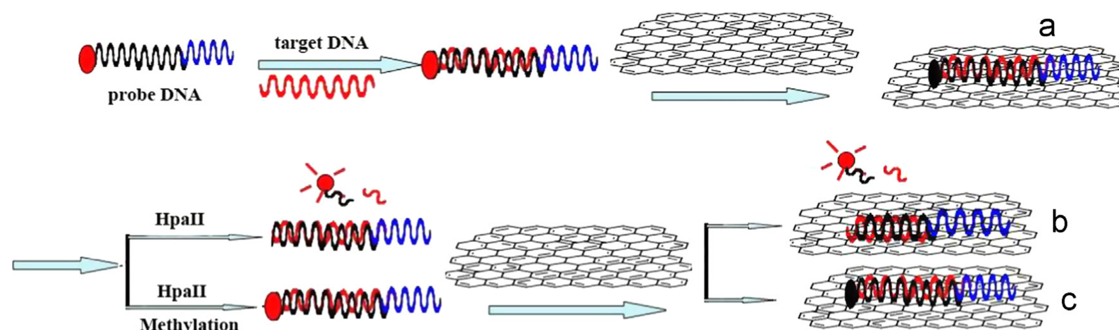
2.4. DNA methylation induced by chemical reagents

25 μL of 1 μM double strands DNA was mixed with 5.0 μM $FeSO_4$, 20.0 μM H_2O_2, 10.0 μM L-ascorbic acid, 3.0 μM ethylene diamine tetraacetic acid (EDTA)–2Na and 100 μM DMSO or CH_3CHO. The solution reacted under N_2 atmosphere. After reaction, the mixture solution was centrifuged to separate chemical reagents from DNA. The followed detection procedure was the same as that for M.SssI methyltransferase.

Table 1
Sequence of used DNA.

Oligonucleotides	Sequences (5′–3′)
Probe DNA	FAM-ACCCGGATAAGATGCTACTTACTCAC
Target DNA	AGCATCTTATCCGGGT
One base mismatched target DNA (T1)	AGCATCTTATCCAGGT
Non-complementary target DNA (T2)	GAACATCATAGCCGGAC

Mismatched base is underlined.



Scheme 1. Fluorescence detection of DNA and DNA methylation based on GO and endonuclease HpaII. .

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