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Amplification strategy for sensitive detection of methyltransferase activity based on surface plasma resonance techniques

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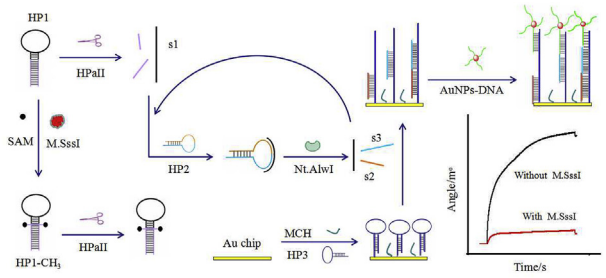
HIGHLIGHTS

- A SPR strategy for sensitive detection of M.SssI activity has been developed.
- Double amplification efficiency of AuNPs and DNA cyclic reaction was utilized.
- The assay of M.SssI activity in human serum samples was realized.
- Inhibition analysis shows potential application of the method in cancer diagnosis and therapeutics.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, a newly developed surface plasma resonance (SPR) system for the sensitive detection of M.SssI activity has been designed based on double signal amplification with DNA chain cyclic reactions and AuNPs. In the absence of M.SssI, hairpin DNA 1 (HP1) can be cleaved into s1 fragments catalyzed by HpaII. The s1 fragments can then trigger a recycling process of hairpin DNA 2 (HP2) hybridization and subsequently release massive s2 and s3 in the solution of Nt.AlwI and HPII. AuNPs-DNA can be captured on gold film by the released s2 and s3 to produce a strong SPR signal. Whereas in the presence of M.SssI, methylated HP1 cannot be cleaved by HpaII, thus produce a weak SPR signal. The SPR signals are dependent on the M.SssI concentration in the range from 0.5 to 50 U/mL. The successful detection of M.SssI activity in clinical serum samples and inhibition of M.SssI using 5-Aza and 5-Aza-dC indicate a great potential of this strategy for building new monitoring platform in bioanalysis and clinical biomedicine.

1. Introduction

In human cells, the methylation of the promoter of cytosine phosphoric guanine (CpG) dinucleotides islands catalyzed by DNA methyltransferase M.SssI is extremely important to lots of normal cellular processes including development, transposon silencing, gene regulation, and so on [1]. Alterations of M.SssI may lead to aberrant DNA methylation, which is related to various pathogenic mechanisms affecting humans [2,3]. M.SssI, therefore, has become an important pharmacological target for the treatment of tumors [4]. Traditional methods including radioactive labeling methylation approaches [5,6], menthyaltion specific polymerase chain reaction [7], restriction enzyme coupled with Southern blot [8], and high

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performance liquid chromatography [9], have been extensively used for M.SssI activity detection. However, these approaches encounter many embarrassed issues, such as the need of radioactive reagents, complicated sample preparations, bulky and expensive instruments. Such limitations have provoked recent development of methods for detecting M.SssI activity including electrochemistry [10,11], electrochemiluminescence [12], photoelectrochemistry [13], fluorescence [14,15], etc. Most of these methods also have shortcomings. For example, fluorometric method needs double labeled DNA fluorometric probes, electrochemical method needs electroactive labels, and the sensitivity of these methods needs to be improved. Therefore, development of sensitive and simple approaches to detect M.SssI activity is highly demanded.

Surface plasmon resonance (SPR) techniques allow for the characterization of molecular interactions in a highly efficient fashion without extra labeling or tagging steps [16], which can avoid potential interference and strenuous labors, comparing with the methods of fluorometry and electrochemistry determination. So SPR techniques have been successfully applied for detecting many chemical and biological samples [17]. Li et al. developed a SPR senor for detecting Dam MTase activity [18]. However, SPR techniques still have been limited by their low sensitivity and high detection limit. To address these drawbacks, amplification of the detection signal is remarkably important. In recent decades, AuNPs have been used to amplify signal in many sensor systems for detecting small molecules [19–23]. But until now, few amplified strategies are available for AuNPs-based SPR techniques to detect M.SssI activity. In addition, only a few highly sensitive sensors have been developed by nicking endonuclease Nt.AlwI-assisted amplification [24,25] for the detection of Dam activity, but no reports are associated with M.SssI activity assay.

Herein, we design a SPR sensor for sensitive and selective detection of M.SssI activity based on DNA chain cyclic reactions and AuNPs dual amplification. In the presence of M.SssI, the DNA chain cyclic reactions are forbidden and therefore generate a weak SPR signal. The SPR signals are dependent on the concentration of M.SssI activity. The biosensor system exhibits high sensitivity and specificity toward the activity of M.SssI. This strategy holds great promise and practical significance for the analysis of M.SssI and shows great potential in anticancer drug discovery.

2. Experimental section

2.1. Materials and reagents

6-Mercapto-1-hexanol (MCH), 1,4-dithiothreitol (DTT), 5-azacytidine (5-Aza), and 5-aza-2′-deoxycytidine (5-Aza-dC) were purchased from Sigma-Aldrich (St.Louis, USA). Trisodium citrate and hydrogen tetrachloroaurate(III) ($HAuCl_4$) were obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Nicking enzyme Nt.AlwI, restriction endonuclease HpaII, 1 × CutSmart Buffer, CpG MTase M.SssI, 10 × NEBuffer 2, and S-adenosylmethionine (SAM) were purchased from New England Biolabs (Ipswich, USA). 20% tetrabromoethane (TBE) gel and oligonucleotides were purchased from Invitrogen Biotech Co. Ltd (Shanghai, China), and the sequences are listed in Table 1. The oligonucleotides were dissolved in 10 mM pH 7.4 Tris-HCl solutions containing 0.1 M NaCl to the desired stock concentrations and stored at -20 °C. Each hairpin DNA (HP) was heated to 95 °C for 10 min and slowly cooled down to room temperature before use. All the thiolated DNA sequences were treated with DTT for 2.5 h and then separated using a Nap-5 column prior to use. All solutions were prepared with ultrapure water (18.2 MΩ cm resistivity) supplied by a Millipore Milli-Q system.

Table 1
Sequences of the used oligonucleotides.

DNA sequence (5′ - 3′)
HP1 TAC↓*CGGTACTGGTCTGATCCTAC*↓CGGTA
HP2 *AACTTATGGATCAGAC*↓CAGACATAATAAGTTGT
HP3 SH-TTTACAACTTATTATGTCTGATCCATAAGTTATAATAAGTTGT
SH-DNA SH-TTTACAACTTATTAT

The portions underlined in HP1 indicate the recognition site of M.SssI, the downward facing arrows in HP1 show the cutting site of the HpaII, and the italic portion in HP1 is the sequence of s1. The portion underlined in HP2 indicates the recognition site of Nt.AlwI, the downward facing arrow in HP2 shows the nicking site of the Nt.AlwI, and the italic portion in HP2 is the sequence of s2 and the other portion is the sequence of s3.

2.2. The buffer solutions employed

(1) HP3 immobilization buffer: 10 mM pH 7.4 Tris-HCl containing 0.1 M NaCl. (2) DNA hybridization buffer: 10 mM pH 7.4 Tris-HCl containing 0.1 M NaCl and 20 mM $MgCl_2$. (3) 1 × NEBuffer 2: 10 mM pH 7.9 Tris-HCl containing 50 mM NaCl, 10 mM $MgCl_2$, and 1 mM dithiothreitol. (4) 1 × CutSmartBuffer: 20 mM pH 7.9 Tris-Ac containing 50 mM KAc, 10 mM $Mg(Ac)_2$, and 100 μg/mL BSA. (5) SPR determination buffer: 0.1 M pH 7.4 phosphate buffered saline (PBS) solution.

2.3. Characterizations and apparatus

SPR measurements were performed on an Autolab Springle instrument (Eco Chemie B.V, The Netherlands). The surface properties of the modified electrodes were characterized by electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) on an electrochemical workstation (Eco Chemie B.V., The Netherlands) using a three-electrode system. The three-electrode system consists of an Ag/AgCl reference electrode, a platinum wire counter electrode, and a gold chip (2 mm diameter) working electrode. The CV curves were recorded in 10 mM pH 7.4 PBS containing 2.5 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl by scanning the potential from −0.1 V to +0.6 V at a scan rate of 50 mV/s. The EIS measurements were performed in the presence of a 2.5 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) mixture in pH 7.4 PBS containing 0.1 M KCl by applying an alternating current voltage of 5 mV amplitude in a frequency range from 0.05 Hz to 104 Hz. The UV-visible (UV-vis) absorption spectra were obtained on a UV-2450 spectrophotometer (Shimadzu, Japan). Scanning electron microscope (SEM) images were recorded using a Quanta 200 SEM (FEI, USA) with an accelerating voltage of 20 kV. Gel electrophoresis (Tanon 3500, Shanghai, China) was carried out to verify the feasibility of the sensing system.

2.4. Synthesis of AuNPs

All glassware were cleaned in a bath of freshly prepared 3:1 HCl/HNO_3 (aqua regia), then rinsed throughout in ultrapure water and dried prior to use. AuNPs (13 nm) were prepared with a slight modification to a classic citrate method [26,27]. 50 mL of 0.01% $HAuCl_4$ solution was heated to boiling with vigorous stirring, and then 1 mL of 5% trisodium citrate solution was added rapidly. When the color of the solution changed from gray yellow to deep red, the resulting colloidal suspension was stirred for 5 min to allow it cool to room temperature. The formed AuNPs solution was stored in a brown glass bottle at 4 °C.

2.5. Preparation of AuNPs-DNA

The initial SH-DNA was activated by DTT for 2.5 h at room