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Proximity-dependent isothermal cycle amplification for small-molecule detection based on surface enhanced Raman scattering

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ABSTRACT

A novel proximity-dependent isothermal cycle amplification (PDICA) strategy has been proposed and successfully used for the determination of cocaine coupled with surface enhanced Raman scattering (SERS). For enhancing the SERS signal, Raman dye molecules modified bio-barcode DNA and gold nanoparticles (AuNPs) are used to prepare the Raman probes. Magnetic beads (MBs) are used as the carrier of amplification template and signal output products for circumventing the problem of high background induced by excess bio-barcode DNA. In the presence of target molecules, two label-free proximity probes can hybridize with each other and subsequently opens the hairpin connector-probe to perform the PDICA reaction including the target recycling amplification and strand-displacement amplification. As a result, abundant AuNPs Raman probes can be anchored on the surface of MBs and a low detection limit of 0.1 nM for cocaine is obtained. This assay also exhibits an excellent selectivity and has been successfully performed in human serum, which confirms the reliability and practicality of this protocol.

1. Introduction

Small molecule detection plays a significant role in physiological function research, drug discovery, and detection of drug residues in foods, etc (Cheng et al., 2012). In this article, cocaine is served as a representative model target for testing new analytical techniques. Cocaine, also known as “cocoa essence”, is one of the most widely abused drugs, and the associated social problems are also very prominent. Commonly used methods for cocaine detection are based on chromatography or mass spectrometry (Bailey, 1994; Rossi et al., 2005, 2008). More recently, a great progress has been made in the construction of cocaine sensors based on the specific recognition of the aptamer for cocaine and a variety of colorimetric (Zhang et al., 2008; Sharma et al., 2012), electrochemical (Baker et al., 2006; X. Li et al., 2008) and fluorescent (Shlyahovsky et al., 2007; He et al., 2010) methods have been developed. These strategies, however, suffer from intrinsic limitations of complicated manipulation, low sensitivity or selectivity, limiting their practical application.

Proximity assay is a newly developed method in recent years and the key concept is “proximity effect”, which depends on the simultaneous and proximate recognition of a target molecule by a pair of affinity probes (Fredriksson et al., 2002; Gullberg et al., 2004; Zhang et al., 2007). Based on this mechanism, proximity ligation assay (PLA) is particularly used for protein detection by employing antibody–oligonucleotide or aptamer pairs conjugates as probes and polymerase chain reaction (PCR) is used as the readout (Fredriksson et al., 2002; Gullberg et al., 2004; Söderberg et al., 2006; Gustafsdottir et al., 2007). Due to the dual recognition, proximity assay has drastically enhanced the sensitivity and specificity of protein detection. So it is very valuable if proximity assay is used in the quantitative detection of small molecules.

Although PCR offers PLA extremely high sensitivity assay, it has many limitations for application, such as complication and time-consuming (Hu et al., 2012). Thus the design of simple readout methods for sensitive proximity assay is in urgent demand. Recently, isothermal cycle amplification is particularly used for biomolecule detection based on scission or replication of nicking endonuclease (Xu et al., 2009), polymerase (Guo et al., 2009) and exonuclease (Zuo et al., 2010). Overall, these detection systems can be operated at a constant temperature, thus no specialized instrumentation is needed and the time is less than that of thermal-cycling techniques. In addition, isothermal cycle amplification can be performed autonomously and repeatedly once trigged by the target, thus large amounts of DNA products can be yielded to enhance the signal, and the sensitivity is significantly increased (Shlyahovsky et al., 2007; D. Li et al., 2007; Weizmann et al., 2008; Wang et al., 2011).

As a nondestructive and noninvasive technique, SERS is of particular interest for biological sensing due to its superiority in

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enormous enhancement of Raman intensity, minimal sample preparation, ease of operation and resistance of photobleaching (Nie and Emory, 1997; Cao et al., 2002; Chen et al., 2010). Up to now, SERS has been successfully applied to analyze some small-molecules (M. Li et al., 2012; Chen et al., 2008), DNA (Jin et al., 2006; Hu and Zhang, 2010; Lim et al., 2011), protein (Wang et al., 2011; Y. Li et al., 2012) and cells (Qian et al., 2008; Ye et al., 2012). Herein, we present the marriage of the proximity assay concept with isothermal cycle amplification to give a simple, rapid and sensitive method, termed proximity-dependent isothermal cycle amplification (PDICA). Then it has been successfully applied in cocaine detection coupled with surface-enhanced Raman scattering (SERS). Combining the simplicity of isothermal cycle amplification and the inherent advantage of SERS, the proposed PDICA method might hold great promise for biomolecule determination in basic research and practical application.

2. Experimental

2.1. Materials and apparatus

Oligonucleotides designed in the present study were synthesized and purified by Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The purity is about 95% and the sequences were listed in Table S1 (see Supplementary materials). Cocaine, herein and caffeine were purchased from the State Narcotic Laborary (Beijing, China). Benzoyl ecgonine, methylecgonine and tetrachloroauric acid ($HAuCl_4 \cdot 4H_2O$) were purchased from Sigma-Aldrich (Shanghai, China). The nicking endonuclease (NEase), Klenow Fragment polymerase (KF polymerase) and the mixture of four dNTPs were purchased from New England BioLabs, Inc. (Beijing, China). Other chemicals employed were of analytical reagent grade and were used without further purification. Magnetic microbeads coated with carboxyl groups (MBs-COOH, particle size: 0.5–1.0 μm) were purchased from Tianjin BaseLine ChroTechResearch Center (China). The gold slide used for Raman detection was purchased from BioNavis Ltd (Finland).

SERS detection was performed on an inVia Raman Microscope (Renishaw, England). Non-denaturing polyacrylamide gel electrophoresis (PAGE) was carried out on the Tanon EPS-300 power supply (Tanon Science & Technology Co., Ltd., Shanghai, China), and the PAGE patterns were imaged on a WD-9413B gel imaging system (Beijing Liuyi Instrument Factory, Beijing, China). UV–vis absorption spectra were carried out on a Cary 50 UV–vis–NIR spectrophotometer (Varian). Transmission electron microscopy (TEM) image was taken with JEOL JSM-6700F instrument (Hitachi).

2.2. Preparation of AuNP-functionalized Raman probe

The AuNP-functionalized Raman probe was prepared as follows: 10 μL of 10^{-7} M primer DNA (S5) and 50 μL of 10^{-6} M Raman dye-conjugated signal DNA (S6) were added into 1 mL of AuNPs (for preparation and characterization of AuNPs, see Supplementary materials) and incubated at 37 °C for 24 h. The DNA-AuNP conjugates were aged in salt solution (0.1 M NaCl, 10 mM acetate buffer solution) for another 12 h. To remove excess oligonucleotides, the mixture was centrifuged at 10,000 rpm for 30 min and the supernatant was removed, leaving the red precipitate at the bottom. Then the precipitate was washed and centrifuged repeatedly for three times. The resulting Raman probe was dispersed into a buffer solution (0.01 mM PBS, pH 8.2, 0.3 M NaCl) and stored at 4 °C for further use. The UV–visible spectra and optimization of the ratio of barcode DNA to signal DNA for Raman probe were shown in Supplementary materials.

2.3. Immobilization of hairpin capture-DNA onto MBs

The procedures for the immobilization of hairpin capture-DNA onto MBs were as follows. An amount of 5 μL of carboxyl-modified MBs suspension was washed with 400 μL of 0.1 M imidazol-HCl buffer (pH 6.8) three times, followed by activation in 0.1 M EDC solution for 30 min. Then 0.1 nmol of hairpin capture-DNA (S4) activated with 20 μL of 30 mM TCEP was added to the activated MBs and the resultant mixture was incubated for 12 h at 37 °C with gentle shaking. The supernatant was removed with magnetic separation, the resulting precipitate was washed with 300 μL of 10 mM pH 7.4 phosphate buffer containing 0.01 M NaCl for three times, and then redispersed in the same buffer solution and stored at 4 °C for further use.

2.4. The sensing procedure

Upon testing various experimental conditions, the cocaine determination could be briefly described as follows: first, 10 μL of cocaine solution of a specific concentration was mixed with proximity probe1, proximity probe2 (10 μL, 5 μM) and the hairpin connector-probe (10 μL, 5 μM). The resultant solution was incubated for 10 min at room temperature to form the quadripartite complex. Subsequently, the prepared hairpin capture-DNA-MBs, 10 μL of Raman probes, 5 μL of dNTPs, 1 μL of NEase and 2 μL of KF polymerase were added to the above solution, followed by incubation at 37 °C for 90 min. Then the reaction was terminated by heating the mixture at 80 °C for 20 min to inactivate the enzymes. Finally, the resulting Raman probe conjugated MBs were washed with 200 μL of 0.01 M PBS (pH 7.0) three times and then resuspended in 50 μL of PBS for SERS detection.

2.5. SERS measurement

The resulting Raman probe conjugated MBs were cast onto the gold slide and air-dried at room temperature before the SERS analysis. Raman measurements were conducted on an inVia Raman microscope with an excitation laser of 633 nm. We use the microscope equipped with a 50× objective to focus the incident excitation laser. The laser power on the sample was 5 mW, the accumulation time was 5 s, and the Raman spectra were calibrated with the WiRE Raman Software Version 3.3 from Renisaw Ltd. Three spectra from different sites was collected from each sample and averaged to represent the SERS results, and three repeated experiments were performed. Error bars showed the standard deviation of three experiments.

2.6. Nondenaturing polyacrylamide gel electrophoresis (PAGE)

The species produced in the process of PDICA were characterized by nondenaturing polyacrylamide gel electrophoresis (PAGE) analysis (Acr=acrylamide, Bis=*N*,*N*′-methylenebisacrylamide; Acr/Bis=29/1). Tris-acetate-EDTA (TAE) (pH=8.5) was used as the separation buffer and the PAGE was generally run at 120 V for 2 h with loading of 9 μL of each sample into the lanes.

3. Results and discussion

3.1. The design principle of the PDICA-based SERS method

Scheme 1 depicts the principle for analyzing the target molecule based on PDICA. The structure of two proximity-probes (S1 and S2) and a connector-probe (S3) used in this method is shown in Scheme 1(A). For designing the proximity-probes, the aptamer binding site of cocaine is split into two aptamer "half-sites"