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Portable aptamer biosensor of platelet-derived growth factor-BB using a personal glucose meter with triply amplified

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ABSTRACT

Sensitive and rapid detection of platelet-derived growth factor BB (PDGF-BB), a cancer-related protein, could help early diagnosis, treatment, and prognosis of cancers. Although some methods have been developed to detect PDGF-BB, few can provide quantitative results using an affordable and portable device that is suitable for home use or field application. In this work, we report the first use of a portable kind of personal glucose meter (PGM) combining a catalytic and molecular beacon (CAMB) system with a cation exchange reaction (CX reaction) for ultrasensitive PDGF-BB assay. It realized the amplification of the detection in three ways, including greater aptamer payload on nanoparticles, CX reaction releasing thousands of Zn^{2+} and the cycle by the catalyzing cleavage of 8–17 DNAzyme. In the process, with the addition of PDGF-BB into the aptasensor, the specific recognition between aptamer and protein was initiated resulting in the combination of ZnS NNC for further CX reaction to release thousands of Zn^{2+}, which could cleave the substrate DNA in the CAMB system realizing multiple cycle. The cleaved DNA fragment was designed with invertase-labeled could convert sucrose into glucose which could be detected and quantified by PGM accompanying with the change of color of the control window from yellow to green. The enhanced signal of the PGM has a relationship with the concentration of PDGF-BB in the range of 3.16×10^{-16} M to 3.16×10^{-12} M, and the detection limit is 0.11 fM. Moreover, the catalytic and cleavage activities of 8–17 DNAzyme can be achieved in solution; thus, no enzyme immobilization is needed for detection. The triply amplified strategy showed high selectivity, stability, and applicability for detecting the desired protein.

1. Introduction

Platelet-derived growth factor-BB (PDGF-BB), an important cytokine in serum, plays a vital role in regulating cell growth and division. It is a necessary protein for increased healing coupled with fibroblast activation and granulation tissue formation in the treatment of some human chronic dermal wounds (Pierce et al., 1995). Because it is close to several horrible diseases such as atherosclerosis, fibrosis and often overexpressed in human malignant tumors serving as an indicator for tumor angiogenesis (Clarke et al., 1984), the recognition and quantification of PDGF-BB are of particular significance in biomedical fields. Until now, various methods have been applied in PDGF-BB determination, such as fluorescence (Qiu et al., 2011), chemiluminescence, colorimetry (Tang et al., 2012) and electrochemistry (Wu et al., 2010). Most of these methods possess high sensitivity and selectivity, but relatively expensive equipment and trained operators are often needed, which made their application limited in remote areas. Therefore, it is highly desirable to develop an easy and portable sensor for sensitive determination of PDGF-BB.

The development of portable sensors for rapid, on-site and cost-effective detection of a broad range of targets has long been sought, because such sensors have the potential to revolutionize scientific research, environmental monitoring and personal healthcare in urban areas in developed countries as well as rural areas in developing countries (Daar et al., 2002; Martinez et al., 2007; Fan et al., 2005; Xia et al., 2010; Zhang et al., 2002; Giepmans et al., 2006; Wegner et al., 2007; Murray et al., 2008; Wang et al., 2010; Favier et al., 2001). Despite their great promise and many years of investigation, only a limited number of sensors are commercially available to the public at present. Perhaps the most successful example of the portable sensor is the personal glucose meter (PGM) (Clark et al., 1962; Montagnana et al., 2009), which is widely available in stores and has either saved or

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improved the quality of lives of millions of diabetic patients worldwide. The broad success of PGMs is largely due to its a great deal of advantages including the portable "pocket" size, cost effectiveness, reliable quantitative results and easy operation (Carroll et al., 2007). In the early stages of the development of PGM, the application of this successful portable sensor is limited in its single target (glucose) and its dynamic range of glucose (Clark et al., 1962; Montagnana et al., 2009). However, recently, researchers have developed various approaches to expand the application of PGM for the detection of different targets. For example, Xiang and Lu (Xiang and Lu, 2011, 2012a, 2012b, 2013) developed sensors to monitor a series of non-glucose targets by taking advantage of invertase-labeled functional DNAs and antibodies. Yan et al. (2013) reported a target-responsive "sweet" hydrogel combined with a commercial glucose meter for cocaine and adenosine triphosphate detection. Su et al. (2013) established a sensitive copper (II) sensor which combined the merits of PGM and click chemistry. In 2013, portable PGM has also been applied to detect PDGF-BB successfully by introducing invertase conjugates to secondary aptamer (Ma et al., 2014). However, due to the PDGF-BB exists at extremely low concentrations in living organisms, the routine aptameric methods hinder further development of PDGF-BB detection in real samples (Zhang and Zhang, 2012). Consequently, there still has been a major challenge to develop sensitive portable methods for the detection of PDGF-BB.

DNAzymes are nucleic acids that isolated from combinatorial oligonucleotide libraries by in vitro selection (Robertson et al., 1990; Breaker, 2000; Wilson and Szostak, 1999; Liu et al., 2009). DNAzymes are not only similar to protein enzymes, exhibiting high catalytic cleavage activity toward specific substrate, but also much more stable than protein enzymes, able to be denatured and restored many times without losing catalytic activity toward substrate and easily produced using a commercial DNA synthesizer (Liu et al., 2009). As one of the most interesting DNAzymes, Zn^{2+}-requiring 8–17 DNAzyme (Zhang et al., 2010; Lu et al., 2011; Jiang et al., 2015), is usually composed of a substrate strand and an enzyme strand. In the presence of Zn^{2+}, the specific cleavage of substrate strand is nicked, resulting in the substrate strand split into two fragments, one of which contains the signal molecules triggering the amplification. Zn^{2+}-requiring 8–17 DNAzyme exhibits many excellent characteristics, such as simple synthesis, good stability, easy labeling, and design flexibility, which enables Zn^{2+}-requiring 8–17 DNAzyme to be particularly attractive in sensing platforms as signal amplification elements for the design of aptasensor that are highly specific for a number of targets such as metal ions and small biological molecules (Liu et al., 2009; Zhang et al., 2011). Recently, by integrating an 8–17 DNAzyme with molecular beacon, a catalytic and molecular beacon (CAMB) amplified system has been reported, realizing the true enzymatic multiple turnover (one enzyme catalyzes the cleavage of several substrates) of catalytic beacons for amplified signal detection. The unique features make 8–17 DNAzyme integrated CAMB strategy very suitable for bioassay applications to amplify the signal.

Ion-exchange reactions based on place-exchanging the cations on the nanoscale ionic material with a different set of cations (Peng et al., 1998) have obtained great attention in chemical analysis, recovery of useful ions, materials synthesis and water purification. In chemical analysis, cation ion-exchange (CX)-based amplification is an effective amplification strategy to improve the sensitivity of assays. It was realized through the incorporation of metal ion with metallic nanoparticles to trigger large amounts of signal reporter unit which was usually from the newly produced ionic nanomaterials with diameter of a few nanometers. They can encapsulate thousands of the corresponding ions resulting in a large signal enhancement in this fast and gentle process. Due to the unique attributes of rapid kinetics at room temperature (orders of magnitude faster than in the bulk), the tuning of reactivity via control of nanocrystal size, shape, surface faceting and the avoidance of strong acids or corrosive oxidants, CX-based amplification has been employed as a convenient tool for sensitive detection of a variety of targets, such as DNA (Zhang et al., 2015a, 2015b, 2015c), microRNA (Li et al., 2009a), femtomolar proteins (Yao et al., 2012a, 2012b), telomerase (Wang et al., 2015), glutathione (Shi et al., 2013) and so on. Zhong's group has reported a series of work on cation-exchange-based fluorescence amplification (Li et al., 2009a, 2009b; Yao et al., 2011, , 2012a, 2012b, 2013). By exchanging nonfluorescent CdSe or ZnS NC clusters with Ag^+, Ag_2Se NCs together with large amounts of the released Cd^{2+} or Zn^{2+} cations were formed. The generated cations can coordinate to metal-responsive fluorophores and further activate fluorogenic dyes. Due to each ZnSe NC, with a calculated diameter of 5.38 nm, contains 2051 Zn atoms (Kucuur et al., 2007), this CX-based amplification method exhibits remarkably high signal enhancement. Owing to the large amounts of Zn^{2+} released through the simple CX reaction and as we know Zn^{2+}could specifically cleave the substrate strand in 8–17 DNAzyme system to amplify the signal, we were inspired to design a system combining the CX with Zn^{2+}-requiring-8–17 DNAzyme.

In this paper, we first report a novel triply amplified PDGF-BB aptasensor with the cost-effective portable PGM as readout. This triply amplified newly PGM platform couples the CAMB system with CX-based amplification. Zn^{2+}-requiring 8–17 DNAzyme was chosen as the catalytic unit due to its high catalytic activity and expanded functionality by the adoption of Zn^{2+} as the cofactor. This strategy can significantly improve sensitivity in three ways. First, nanoparticles have large surface areas, providing greater aptamer payload. Second, each ionic nanocrystal can release thousands of Zn^{2+} into solution by CX reaction. Third, one 8–17 DNAzyme can catalyze the cleavage of several substrates. Therefore, after the CX reaction, the CAMB system affords an amplified signal through cycling and regenerating the Zn^{2+}-dependent 8–17 DNAzyme to realize multiple enzymatic turnovers. The proposed portable aptasensor has been applied to detect PDGF-BB in clinical human saliva samples with satisfactory results. It is worth noting that the operating principle of this study could be extended as a general protocol for other targets of interest.

2. Experimental procedures

Materials, Apparatus and the electrochemical experimental process are in Supplemental information.

2.1. DNA-invertase conjugation

The conjugation procedure was similar to previous work with minor modifications (Xiang and Lu, 2011, 2012a, 2012b; Zhou et al., 2014). Briefly, 30 μL of 1 mM sDNA, 2 μL of 0.1 M buffer A, and 2 μL of 30 mM TCEP were mixed and kept at room temperature for 1 h. After that, the excess TCEP was removed by Amicon-3K using buffer A (0.1 M NaCl, 0.1 M sodium phosphate buffer, pH 7.4, without 0.05% Tween-20) for 3 times. Meanwhile, 1 mg of sulfo-SMCC was mixed with 400 μL of 20 mg/mL invertase in buffer A. After vortexing, the solution was placed on a shaker for 1 h at room temperature. Then, the mixture was centrifuged to remove excess insoluble sulfo-SMCC. The supernatant was washed 8 times by Amicon-100 K using buffer A. The above solutions of TCEP-activated sDNA and sulfo-SMCC-activated invertase were mixed and kept at room temperature for 48 h. To remove unreacted sDNA, the solution was purified by Amicon-100 K using buffer A over five wash cycles.

2.2. Synthesis of ZnS Nanocrystal Clusters (NCCs) and Fe_3O_4 NPs

The ZnS NCCs were synthesized by a hydrothermal approach, using thiourea NH_2CSNH_2 to control cluster growth. In a typical synthesis, 0.8 mmol of $Zn(Ac)_2{\cdot}2H_2O$ and 20 mmol of thiourea were dissolved in 20 mL of ultrapure water to form a clear solution after being stirred for 30 min at room temperature. The solution was then transferred into a