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A reusable aptamer-based evanescent wave all-fiber biosensor for highly sensitive detection of Ochratoxin A

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ABSTRACT

Although aptamer-based biosensors have attracted ever-increasing attentions and found potential applications in a wide range of areas, they usually adopted the assay protocol of immobilizing DNA probe (e.g., aptamer, aptamer-complementary oligonucleotides) on a solid sensing surface, making it critical and challengeable to keep the integration of nucleic acid surface during the regeneration and the restoration to its original DNA probe form after repeated uses. In order to address the issue, we report a novel aptamer-based biosensing strategy based on an evanescent wave all-fiber (EWA) platform. In a simple target capturing step using aptamer-functionalized magnetic microbeads, signal probes conjugated with streptavidin are released and further detected by a EWA biosensor via a facial dethiobiotin–streptavidin recognition. Apart from the inherent advantages of aptamer-based evanescent wave biosensors (e.g. target versatility, sensitivity, selectivity and portability), the proposed strategy exhibits a high stability and remarkable reusability over other aptasensors. Under the optimized conditions, the typical calibration curve obtained for Ochratoxin A has a detection limit of 3 nM with a linear response ranging from 6 nM to 500 nM. The dethiobiotin–streptavidin sensing surface instead of the traditional nucleic acid one can be reused for over 300 times without losing sensitivity.

1. Introduction

By virtue of their high sensitively, fast kinetics, high selectivity, and facial synthesis (Haun et al., 2010; Song et al., 2010; Zhang et al. 2011), DNA-based biosensors have attracted much attention and witnessed their wide application from diagnostics to basic research (Iliuk et al., 2011; Lee et al. 2010; Li et al., 2010; Shao et al., 2012; Wang 2006). Wherein, the aptamer-based biosensor technology is a rapidly developing area which is anticipated to be competitive with immunoassays and other analytical counterparts currently in use (Citartan et al., 2012; Han et al., 2010; Zhou et al., 2010). Aptamer is a single-stranded oligonucleotide that has been screened through an in vitro selection process called SELEX (Systematic Evolution of Ligands by Exponential Enrichment) (Ellington and Szostak, 1990). As a biological recognition element, aptamer is a promising substitution for antibody because of its clear advantages such as simple production, easy storage, good reproducibility and particularly target versatility (e.g., ranging from small organic molecules to heavy metals, proteins, cells, and even intact viral particles) (Minunni et al., 2004; Tombelli et al., 2007; Zhang et al. 2011). Up to now, numerous optical (Spiridonova and Kopylov, 2002; Zhu et al., 2006), electrochemical (Baker et al., 2006; Wen et al., 2011; Wu et al., 2010), and other aptamer-based (Basnar et al., 2006; Li et al., 2007; Liss et al., 2002) sensors have been developed.

Among them, evanescent field fluorescent biosensors have received widespread attention due to their easy access to miniaturization, highly-sensitive and selective sensing (Leung et al., 2007; Wang and Wolfbeis, 2013), wherein the optical fiber is adopted as one of the most promising transducers. In this transducer, light is propagated down the optically denser medium of fiber core by the total internal reflection (TIR), thus generating an electromagnetic wave (i.e., evanescent field) at the interface of the fiber core and the adjacent less dense medium (e.g. aqueous solution samples). The amplitude of the evanescent wave decreases exponentially with distance into the lower refractive index material, which provides the selectivity to only excite the fluorophores adsorbed, adhered, or bound to the fiber surface. The exponential decay of field strength essentially confines transducible optical signals to within a discrete distance from the waveguide's surface (usually with effective depth of 100–200 nm) (Long et al., 2014), minimizing interference or contribution from components in the bulk phase of lower index medium, thus more sensitive to the targets (Taitt et al., 2005; Zhou et al. 2014). Furthermore, the

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excited light is totally reflected away from the detection region, therefore making easily discriminating the fluorescence signal from the excited light and achieving high sensitivities and low detection limits (Farré et al., 2009; Golden et al., 1992; Taitt et al., 2005; Wadkins et al., 1998). Due to the many advantages, researches on the aptamer-based evanescent wave fluorescence biosensors are blooming in very recently (Long et al., 2014; Yildirim et al., 2012).

Like other biosensors, aptasensors are usually in the form of electrodes, chips, and crystals; hence, immobilization of DNA probe (e.g., aptamer, aptamer-complementary oligonucleotides) on the solid phase is essential in the design of aptamer sensors (Bier et al., 1997; Chuang and Shih, 2001; Liu and Tan, 1999; Piunno et al., 1995; Wu et al., 2007; Zhai et al., 1997), which ensures higher sensing sensitivity and easier device integration compared with homogeneous strategies (Balamurugan et al., 2008; Cosnier and Mailley 2008). Theoretically, device reusability is one of the natural benefits that come with the immobilization strategy, which enables the device easy to achieve portability, automation, and simplicity of use. Moreover, reusability of biosensor is a critical premise to realizing accurate quantification if the chip is not as cheap as a disposable one. However, some biological activity or appropriate orientation of those aptamers might be lost during/after the localization and other regenerating steps, making direct immobilizing protocols disfavored for reusable detections. Many strategies have been reported to meet this challenge, including the use of concentrated salt solutions (Radi et al., 2005), acid/basic solution (Bier et al., 1997; Dave et al., 2010; He et al., 2013; Minunni et al., 2004; Schlensog et al., 2004; Wu et al., 2007), temperature (Freeman et al. 2009; Piunno et al., 1995), chelating agents such as EDTA (Liss et al., 2002), surfactants such as 90% formamide in a TE buffer (10 mM Tris–HCl, pH 8.3, 1 mM EDTA) (Liu and Tan, 1999), sodium dodecyl sulfate (Lai et al., 2007), and thiol-disulfide exchange chemistry (Moore et al., 2007). In all cases above, the typical procedure for biosensor regeneration is to wash aptamers first until the bound target species are removed, then treatment with the regeneration solution, followed by a final rinse with buffer (Balamurugan et al., 2008). Although much effort has been devoted into the development of reusable aptamer-based biosensors, there are few platforms robust enough to be reused for more than 15 times. The restoration to the original DNA probe form is admitted difficult after repeated uses (Liu and Tan, 1999); therefore, how to improve the reusability of aptamer-based biosensor becomes one of the biggest challenges pending for solving in future.

Motivated by the above studies, here we report a novel aptamer-based evanescent wave all-fiber (EWA) biosensing strategy. This strategy is based on aptamer-modified magnetic beads (MB-Ap), STV-conjugated aptamer-complementary DNA oligonucleotides as signal probes (Sp) and a dethiobiotin-modified fiber which is embedded inside the evanescent wave biosensor system. In a simple target capturing step, Sp are released and further detected by a EWA biosensor via a facial dethiobiotin–streptavidin recognition. Therefore, apart from the inherent advantages of aptamer-based evanescent wave biosensors (e.g. target versatility, sensitivity, selectivity and portability), the proposed technique also exhibits high reusability over other aptasensors. To testify the detection protocol, Ochratoxin A (OTA), a widespread food contaminant which showed neurotoxicity, liver toxicity, renal toxicity, teratogenicity and immunetoxicity (Hayat et al., 2013; Rhouati et al., 2013), was chosen as the analyte.

2. Experimental

2.1. Chemical and reagents

Carboxyl-coated magnetic beads (BioMag® Plus Carboxyl, Catalog Code: BP618, 1.5 μm in average diameter) were purchased from Bangs Laboratories Inc. (Fishers, IN). Amicon-3K/10K/50K centrifugal filters were purchased from Millipore Inc. (Billerica, MA). Glutaraldehyde, Streptavidin (STV), Carbodiimide (EDAC), Sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC), 3-Aminopropyl-triethoxysilane (APTS), Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), Ochratoxin A (OTA), Aflatoxin B1 (AFT B1), Aflatoxin B2 (AFT B2), Deoxynivalenol (DON) and Chloramphenicol (CHL) were purchased from Sigma–Aldrich, Inc. (St. Louis, MO). Other chemicals used for buffers and solvents were purchased from J&K, Inc.

The following oligonucleotides were purchased from Takara Biotechnoly (Dalian, China) Co., Ltd (from left to right: 5′ to 3′):

DNA for immobilization onto magnetic beads (Aptamers, Ap):
NH_2-AAAAAAGATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA
NH_2-AAAAAAGATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA-FAM
DNA for signal probe (Sp) synthesis (Thiol-DNA-Cy5.5):
SH-AAAAAAAAAAAATGTCCGATGCTC-Cy5.5
Buffers used in this work:
Imidazole Buffer: 0.1 M 1-Methylimidazole, pH 7.0
Prehybridization Buffer: 0.1 M Tris, 0.005 M EDTA, 0.5% N-Laurylsarcosine, 1% BSA, pH 7.4
Hybridization Buffer: 10 mM Tris, 1.0 mM EDTA, 0.01% Tween-20 and 1 M NaCl
Buffer A: 0.1 M NaCl, 0.1 M sodium phosphate buffer, 0.05% Tween-20, pH 7.3
Buffer B: 10 mM PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4), 4 mM $MgCl_2$, pH 7.4,
Wash buffer: 0.5% SDS, pH 1.9

All buffers were prepared either in DEPC-treated deionize water or biomolecule grade deionize water (RNA Nuclease, DNA Nuclease free).

Fluorescence spectrophotometer used in this study: Hitachi F7000 (Hitachi Ltd., Japan)

2.2. Preparation of functional magnetic beads

800 nM aptamer (Ap) and 0.1 M EDC were gently mixed in 2 ml imidazole buffer. The mixture were immediately incubated with 20 mg carboxyl magnetic beads (MBs) at room temperature for 24 h to allow the formation of MB-Ap. At the end of the coupling reaction, MB-Ap were treated with prehybridization buffer at 68 °C for 4 h to block unreacted amino groups on the surface. 2 mg synthesized Sp (see supplementary material for the synthesis and PAGE characterization of the signal probes) were added to washed MB-Ap in 2 ml hybridization buffer, and the mixture were kept on a rotator for 2.5 h at room temperature for the formation of MB-Sp. After hybridization, MB-Sp were washed and preserved in 2 ml PBS buffer (10 mM), diluting the final solids content to 10 mg/ml.

2.3. All-fiber evanescent wave biosensing platform

Scheme 1B presents the schematic of a EWA biosensing platform which has been proposed as our previously described (Long et al. 2008) with a significant modification. Briefly, a 635 nm, 10 mW pulse diode laser with a pigtail was coupled into a multimode fiber probe through an optical fiber bundle. The incident light propagated along the fiber probe via TIR. The evanescent