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Triple functional DNA–protein conjugates: Signal probes for Pb^{2+} using evanescent wave-induced emission

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ABSTRACT

We describe here a Pb^{2+}-dependent DNAzyme-based evanescent wave-induced emission (EWIE) biosensing platform using triple functional DNA–protein conjugates as signal probes for Pb^{2+} detection. Upon reaction with Pb^{2+}, the substrate strand is cleaved, releasing an invasion fragment, which is then hybridized with the complementary DNA strand immobilized on magnetic beads, while dissociating of the original hybridized signal probes. The signal probes, consisting of a streptavidin moiety and a Cy5.5 labeled DNA moiety, act simultaneously as signal conversion, signal recognition and signal report elements. Detection of the signal probes is accomplished by first adsorbing to the desthiobiotin-modified optical fiber, followed by fluorescence emission induced by an evanescent field. A linear calibration was obtained from 20 nM to 800 nM with a detection limit of 1 nM. The optical fiber system is robust enough for 250 sensing cycles and can be stored at room temperature over one month. These results demonstrate that application of DNA–streptavidin conjugates has been extended to DNAzyme-based biosensors, maintaining activity, specificity, regeneration and long-term storage ability.

1. Introduction

Rapid detection of specific heavy metal ion has received much attention in scientific research. Of particular interest has been the detection of lead ion (Pb^{2+}), a dangerous pollutant which causes adverse health effects in people, including renal malfunction, delayed physical/mental development, blood–brain barrier, and kidney disease (Chow et al., 2005; Needleman, 2004; Sane et al., 2013). Various instrumental analytical techniques for Pb^{2+} detection have been developed, such as AAS (Ucar et al., 2014), ICP-AES (Hao et al., 2015), and ICP-MS (Palmer et al., 2006). However, these widely-used instrumental techniques are rather complex, requiring sophisticated instrument and professional personnel, which prevent their further application. Therefore the development of new sensing techniques for Pb^{2+} quantification is a hot topic in analytical chemistry in recent years (Deibler and Basu, 2013).

Among newly developed sensors, DNAzyme-based biosensors are particularly attractive (Lan and Lu, 2012; Li and Kong, 2013). DNAzymes, a series of functional oligonucleotides, are able to catalyze several types of reactions and are highly special for metal cofactors such as Pb^{2+} ions (Li et al., 2014), Mg^{2+} ions (Elbaz et al., 2010), UO_2^{2+} ions (Liu et al., 2007), and Zn^{2+} ions (Qian et al., 2014), etc. DNAzymes are stable, low cost, and have selectivities that are comparable with that of antibodies (Li et al., 2000), thus exhibiting strong competitiveness in sensing applications (Willner et al., 2008). Over the past two decades, various DNAzyme-based biosensors for Pb^{2+} using different signal transduction modes have been developed, including colorimetry (Liu and Lu 2003), fluorescence (Zhao et al., 2011), surface plasmon resonance (SPR) (Pelossof et al., 2012), surface-enhanced Raman scattering (SERS) (Wang and Irudayaraj, 2011) and electrochemistry (Xiao et al., 2007). These DNAzyme-based biosensors require simpler sensing instrument and less professional personnel than traditional instrumental techniques.

Many reported DNAzyme-based biosensors operate in homogeneous solution phase. However, moving to surface-based sensors have several inherent advantages. First, surface-based sensors are more amenable to washing, thus minimizing the background noises that challenge most homogeneous sensors (Swearingen et al., 2005). Second, signal enrichment via solid-phase binding is technically easier to achieve, which can lead to ultrasensitive detections. Third, the surface-based sensors exhibit the potential of miniaturization of multifunctional integrated devices, which pave the way toward multi-target sensing platform. Up to now, numerous surface-based sensors have been developed (Zhang and Da, 2010), among them, of particular interest is the evanescent wave induced emission (EWIE) sensing platform (Fig. S1), which is portable, biological compatible, and highly sensitive with a background suppression design (Xiong et al., 2014; Zhong et al., 2014).

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One challenge in developing surface-based sensors is to establish a regenerable sensing surface and keep the biological activities of DNA strands. Aiming at this problem, we use special designed DNA–protein conjugates as signal probes to skirt the problem of direct DNA-immobilizing protocols. Because functional DNAs are selective toward nucleic acid relative events (Breaker, 2002; Seetharaman et al., 2001), while the coupled proteins may serve as amplifying or catalytic units (Sacca and Niemeyer, 2011), DNA–protein conjugates are especially useful in analytical chemistry for target recognition, signal transformation and signal amplification (Tran et al., 2013; Zhou et al., 2014b). Examples include DNA-directed immobilization (DDI) (Wacker et al., 2004), biochip technologies (Niemeyer, 2007), active biocatalytic cascades (Freeman et al., 2009), signal amplifying designs (Mor-Piperberg et al., 2010), and signal transforming using glucose meters (Xiang and Lu, 2012). We once reported a highly reusable aptasensor using DNA–protein conjugates (Wang et al., 2015). Here we incorporate DNAzyme-based designs into the EWIE sensing platform using DNA–streptavidin (STV) conjugates as signal probes. The DNA–STV conjugates work simultaneously as signal conversion, recognition, and report elements, acting as a beneficial complement to the design of a reusable Pb^{2+} biosensor with high sensitivity and selectivity. Besides, DNAzymes provide a further facet of selectively evolved nucleic acids while reducing the cost. The whole detection is rapid and could be conducted at room temperature. In this work, the dethiobiotin-modified sensing surface could be regenerated for about 250 times with less than 6% signal attenuation. To the best of our knowledge, this study is the first to report a reusable EWIE sensor using DNA–protein conjugates and DNAzymes, and expands the scope of application of DNA–protein conjugates to DNAzyme-based sensing.

2. Experimental section

2.1. Materials

Carboxyl-coated magnetic beads (Dynabeads® MyOne™ Carboxylic Acid, Catalog Code: 65012, 1.0 μm in average diameter) were purchased from Invitrogen Dynal AS (Oslo, Norway). Amicon-3K/50K centrifugal filters were purchased from Millipore Inc. (Billerica, MA). Magnetic separation stand (twelve-position) was purchased from Promega (Madision, WI, USA). Glutaraldehyde, desthiobiotin, streptavidin (STV), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC), 3-aminopropyl-triethoxysilane (APTS), and Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Other chemicals used for buffers and solvents were purchased from J&K, Inc. All solutions were prepared using molecular biology grade USP sterile purified water (RNase-, DNase- and protease- free).All oligonucleotides were purchased from Takara Biotechnology (Dalian, China) Co., Ltd. The oligonucleotides were annealed at 90 °C for 30 min in buffers before use. See Supplementary information for DNA sequences, buffers and the optical fiber used in this work.

2.2. Synthesis of signal probes: DNA–STV conjugation

Various methods for covalent/noncovalent coupling of synthetic DNA oligomers to proteins have been described (Niemeyer, 2007). While a number of methods for conjugating DNA with proteins are based on the remarkable biomolecular recognition of biotin by the homotetrameric proteins avidin or streptavidin (Xiang and Lu, 2013), it may introduce unnecessary interference to our sensing system, since the STV moiety of the conjugate is designed to be captured by the desthiobiotin-modified optical fiber, better not to react with biotin in advance.

Therefore the signal probes (DNA–STV conjugates) used in this work were synthesized by conjugating signal probe (Sp) DNA and STV using the heterobifuntional linker, sulfo-SMCC (Xiang and Lu, 2011). Add 0.7 μl of 1 M sodium phosphate buffer (pH 5.5) and 0.7 μL of 30 mM TCEP in sterile purified water to 10 μL of 1 mM Sp DNA (mercapto-terminated) in sterile purified water, then the obtained mixture was kept at room temperature. 1 h later, the mixture was purified by Amicon-3K using buffer A by 8 times. Meanwhile, 0.4 mg sulfo-SMCC and 3 mg STV were added to 150 μL of buffer A. After votexing for 5 min, the solution was rotated for 1 h at room temperature. After that, the excess insoluble sulfo-SMCC was removed by centrifugation, then the obtained clear solution was collected and purified by Amicon-50K using buffer A by 8 times. The purified solution of sulfo-SMCC-activated STV was mixed with the above solution of Sp DNA. The resulting solution was kept at room temperature for 48 h. To remove unreacted thiol-DNA-Cy5.5, the solution was purified by Amicon-50K 8 times using buffer A. The prepared signal probes were preserved in buffer D at 4 °C until use, with the final protein content diluted to 0.5 mg/mL.

2.3. Evanescent wave-induced emission (EWIE) biosensing platform

Fig. S1 presents the schematic of the EWIE biosensing platform which has been proposed as previously described with a moderate modification (Long et al., 2008). Briefly, all samples were pumped into a glass flow cell through a flow delivery system. An optical fiber was embedded inside the cell, along which the incident laser (635 nm) propagated via total inner reflection, thus generating an evanescent field at the fiber surface. The surface of the optical fiber could be functionalized with different molecules or nanomaterials according to actual experimental needs, such as antigens (Long et al., 2010), antibodies (Koets et al., 2014), DNA strands (Long et al., 2011), and other nanomaterials (Liu and Tan, 1999). In order to build a reusable sensing surface, a desthiobiotin-modified fiber was used in this work, see Supplementary information for its preparation. Once being functionalized, the surface was able to capture signal probes (fluorophores modified) within the generated evanescent field. Naturally, fluorophores within this evanescent field would be excited, then the emitted fluorescence was further collected by the same optical fiber, passed through an optical fiber bundle, filtered subsequently by a band pass filter and detected by photodiodes through a lock-in amplifier. One built-in computer worked for the control of delivery system, data acquisition and auto-processing, all mentioned above.

In order to optimize the sensing performances, there were three important changes on the flow cell used in this system (see Fig. S2, Supplementary information for the design of the flow cell). On the basis of the original merits of this system, *i.e.*, reduced optical components and easy optical alignment (Hao et al., 2014; Zhou et al., 2014a), this new EWIE biosensing platform was designed to have better sealing property, less reagents consumption, and ease to standardized production, thus enabling better detection performances for test samples.

2.4. Procedures for Pb^{2+} detection using the EWIE sensing platform

2.4.1. Sensor preparation

A 0.1 mL portion of 10 mg/mL solution of carboxyl magnetic beads (MBs) in a microtube was placed in a magnetic separation stand for about 1 min. Then the clear solution was discarded. The use of magnetic beads was advantageous because of their chemical stability, ease of surface modification, and convenience for separation (Centi et al., 2007; Jinghua et al., 2014). 40 mg of EDC was added to 2 mL buffer B containing 5.8 nM amine-modified DNA.