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Electrochemical aptasensor using the tripropylamine oxidation to probe intramolecular displacement between target and complementary nucleotide for protein array

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ABSTRACT

Tripropylamine (TPA) has different oxidation efficiency at double stranded (ds)-and single stranded (ss)-DNA-modified electrodes. Using this property, a simple but sensitive biosensor using TPA oxidation to probe the intramolecular displacement was constructed with the analysis of lysozyme as model for the first time. After the complementary ss-DNA strand of anti-lysozyme aptamer was immobilized onto gold electrode via gold–thiol bond, the incubation with the aptamer resulted in the formation of ds-DNA. Lysozyme (in 10 μL sample) binding with aptamer displaced the complementary strand because of the high affinity of lysozyme and its aptamer, corresponding to the dissociation of the ds-DNA. The modified electrode was swept in 20 mM TPA solution from 0.2 to 0.95 V. The difference in oxidation current was used to quantify the content of lysozyme with a linear range from 1.0 pM to 1.1 nM. That means 10 amol or 6.0×10^6 lysozyme molecules can be detected. Because the signal is produced from the preconcentrated TPA at the electrode surface, the high sensitivity is achieved over the single site labelling strategy. The proposed method is simple, stable, specific, and time-saving while the complicated sample pre-treatment and the labelling to the DNA strand are avoided. The biosensor was validated by the analysis of the diluted egg white sample directly. The recovery and reproducibility were 93.3–100% and 1.4–4.2%, respectively.

1. Introduction

High-throughput proteome analysis is an important goal after the drafting of the human genome. Because of the simple construction, many different designs have been advanced by the use of high affinity aptamers for the development of bioassay probes (Kerman et al., 2008; Odenthal and Gooding, 2007). Among all the detection systems, the electrochemical methods for the biosensor-based proteome analysis are simple and sensitive, and can be classified into two categories, i.e. label-free and labelling approaches (Willner and Zayats, 2007; Kerman et al., 2008). Willner and Zayats (2007) reviewed the electronic aptasensors extensively. Although each strategy has some distinct advantages, each also presents its own unique limitations. Label-free method based on the electrochemical oxidation of guanine bases is much simple and versatile (Heng et al., 2005). However, the signal is generated regardless whether it is hybridized with the immobilized strand or is non-specifically adsorbed onto the electrode. The high background signal inevitably increases the limits of detection. Electrochemical impedance spectroscopy (EIS), as the measurement of changes in the faradic impedance, suffered the increase in resistance owing the repulsion of ferricyanide by the negatively charged DNA duplex (Odenthal and Gooding, 2007; Xu et al., 2006).

The labelling methods generate the signal from electrochemical active molecules, which either intercalate into the Watson–Crick base pairs of a DNA duplex (Yin et al., 2009b; Carter and Bard, 1990; Jiang et al., 2004; Li et al., 2007a; Wang et al., 2005; Huang et al., 2007; Tansil et al., 2005; Gao and Tansil, 2009; Wang et al., 2009) or bind to the single-stranded DNA (ss-DNA) (Xiao et al., 2005, 2009; Huang et al., 2008; Deng et al., 2009b; He et al., 2007). Those methods are sensitive and selective over the label-free strategy. The conformation alteration during the aptamer-target binding provides a simple approach for aptasensor design by the use of ss-DNA strand labelled with active molecules (Xiao et al., 2005, 2009; Huang et al., 2008; Deng et al., 2009b; He et al., 2007). However, the attachment of an electrochemically active molecule to the end of the immobilized DNA sequence suffers the false positive signal because of the flexibility of the ss-DNA (Xiao et al., 2005, 2009). A ca. 30% signals upon the saturated target levels was observed even in the absence of the target protein (Xiao et al., 2009). The use of intercalated probes provides a simple design to report the recognition and binding of aptamer to its target molecules (Yin et al., 2009b; Carter and Bard, 1990; Jiang et al., 2004; Li et al., 2007a; Wang et al., 2005; Huang et al., 2007; Tansil et al., 2005; Gao and

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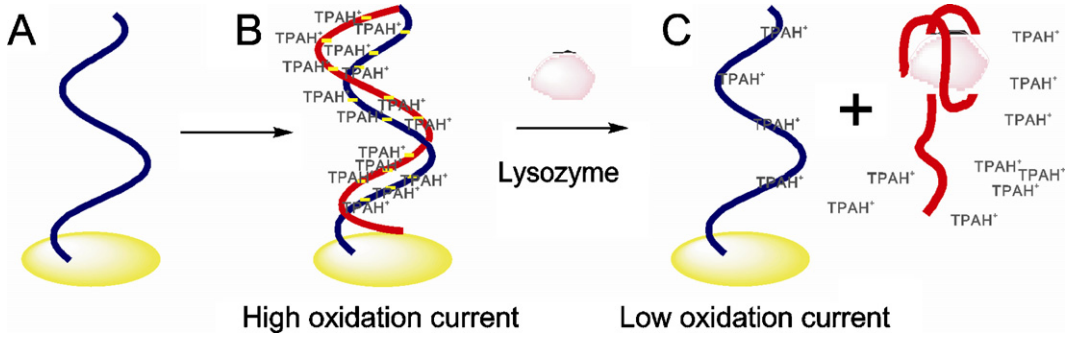

Scheme 1. Schematic illustration of the principle of the aptasensor for the detection of lysozyme, including: the attachment of ss-DNA onto Au electrode; the formation of ds-DNA between the ss-DNA and its complementary strand, anti-lysozyme aptamer; and the hybrid between lysozyme and its aptamer results in the dissociation of ds-DNA. The different oxidation behavior of TPA at ds-DNA- and ss-DNA-modified electrode was used to probe lysozyme.

Tansil, 2009; Wang et al., 2009). However, the labelling techniques are time-consuming since the coupling of the active molecules to the DNA strand is needed. Moreover, the labelling step creates subtle variation in the binding affinities, conformational changes and associated kinetics of the biochemical reagents (Sadik et al., 2009).

If some probing case can be used to indicate the change between ss-DNA and double-stranded DNA (ds-DNA), it can also be used to probe the target analyte, such as the electrostatic interaction between the anionic phosphate backbone and cathodic probes (Cheng et al., 2007; Shen et al., 2007; Chakraborty et al., 2009; Zhang et al., 2006; Nutiu and Li, 2003). $Ru(NH_3)_6^{3+}$, as one of popular cathodic probes, was used to probe lysozyme (Cheng et al., 2007), adenosine (Shen et al., 2007; Chakraborty et al., 2009), to quantify DNA surface density (Zhang et al., 2006), and to monitor DNA hybridization (Nutiu and Li, 2003). The binding of DNA to its target results in the release of adsorbed $Ru(NH_3)_6^{3+}$ and the changed electrochemical signals (Cheng et al., 2007; Shen et al., 2007; Chakraborty et al., 2009; Zhang et al., 2006; Nutiu and Li, 2003).

The ds-DNA strand attached onto the electrode surface can improve the tripropylamine (TPA) oxidation significantly through the electrostatic interaction between the anionic phosphate backbone and the deprotonation of $TPAH^+$, but ss-DNA cannot (Yin et al., 2009b; Pittman and Miao, 2008). Herein, the TPA oxidation responding to the change from ds-DNA to ss-DNA was used to construct a simple biosensing platform for protein assay for the first time. Different to the simple adsorption to $Ru(NH_3)_6^{3+}$, TPA cannot only be adsorbed onto the anonic DNA backbone, but has a high oxidation efficiency within the DNA micro-environment. The procedure for the analysis of lysozyme is illustrated in Scheme 1. After the complementary strand of anti-lysozyme aptamer was immobilized on Au electrode, the introduction of the aptamer achieved the formation of hybrid ds-DNA. Upon target binding with aptamer, the formed ds-DNA was dissociated. The decreased oxidation current of TPA was related to the intramolecular displacement of lysozyme to the complementary strand of its aptamer. Because the signal is from the dissociation of ds-DNA, the strategy is as simple as the label-free methods. Moreover, the signal from TPA oxidation makes the method sensitive and selective as shown in the labelling methods. This strategy combines the advantages of labelling and label-free methods and provides a solution to problem of the need for conformational change for the labelling methods.

2. Materials and methods

2.1. Reagents

Lysozyme was prepared in Tris–HCl buffer (4.7 mM NaCl, 0.56 mM Tris–HCl, 0.14 mM KCl, pH 6.5). All oligonucleotides were prepared to 5 μM with TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.5). The stock solutions of TPA were prepared with doubly distilled water. 2-Mercaptoethanol (MCE) used to block the sensing interface for detection, was obtained from Yangguang Yunneng Biotechnology Company, Tianjin, China. DNA oligonucleotides were synthesized by Takara Biotechnology (Dalian, China). The sequences of the four oligomers used were:

1: 5′-(SH)-$(CH_2)_6$-GCA CTC TTT AGC CCT GAT GAA TTC GTA GAT-3′ (**SS1**)

2: 5′-ATC TAC GAA TTC ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3′ (**SS2**)

3: 5′-(SH)-$(CH_2)_6$-ATC TAC GAA TTC ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3′ (**SS3**)

4: 5′-GCA CTC TTT AGC CCT GAT GAA TTC GTA GAT-3′ (**SS4**)

2.2. Instrumentation

The electrochemical measurements were carried out on a Model LK98BII microcomputer-based electrochemical analyzer (Tianjin Lanlike High-Tech Company, Tianjin, China) using a standard three-electrode system with Ag/AgCl/KCl(sat) electrode as the reference electrode, 2 mm diameter DNA-modified Au electrode as the working electrode, and a Pt wire as the counter electrode. Measurement was carried out using the potential scanned linearly using three-electrode system with bare or DNA-modified gold electrodes as the working electrode.

2.3. Determination of lysozyme using TPA oxidation to probe the dissociation of ds-DNA

The new gold electrode was polished with 0.3- and 0.05-μm aluminum slurry and ultrasonicated with distilled water for 15 min. It was electrochemically cleaned in 1 M H_2SO_4 via potential scanning between −0.2 and 1.6 V until the stable voltammetric peaks were obtained. For the determination of lysozyme, the clean electrode was soaked in 5 μM thiolated DNA (**SS1**) to prepare ss-DNA-modified electrode for 1.5 h at 36 °C. 10 μL of 0.1 M MCE solution was dropped onto the electrode surface and was incubated for 1.5 h at 36 °C in order to block the sensing interface. The modified electrode was soaked in 5 μM complementary ss-DNA, **SS2**, to form ds-DNA on the electrode surface. 10 μL lysozyme sample with various concentrations was dropped on the electrode surface for 1 h at 36 °C. The hybridization of lysozyme and its aptamer resulted in the dissociation of ds-DNA formed between **SS1** and **SS2**. After the modified electrode was cleaned thoroughly and kept into the TPA-containing solution and stirred 15 s for the adsorption equilibrium of TPA on the modified electrode, the potential applied to the potentiostat was scanned linearly from 0.2 to 0.95 V versus Ag/AgCl for the electrochemical determination of lysozyme. As a compar-