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

Dong-Yuan Liu, Yue Zhao, Xi-Wen He, Xue-Bo Yin *

Research Center for Analytical Sciences, College of Chemistry, Nankai University, 94 Weijin Road, Tianjin 300071, China

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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

* Corresponding author. Fax: +86 22 25302458.
E-mail address: xbyin@nankai.edu.cn (X.-B. Yin).

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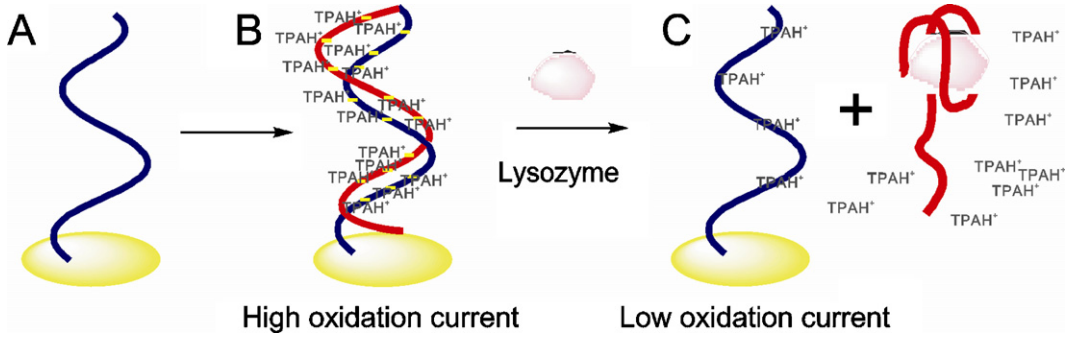

Scheme 1. Schematic illustration of the principle of the aptasensor for the detection of lysozyme, including: the attachment of ss-DNA onto Au electrode; the formation of ds-DNA between the ss-DNA and its complementary strand, anti-lysozyme aptamer; and the hybrid between lysozyme and its aptamer results 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-

ison, anti-lysozyme aptamer, **SS3**, was first immobilized onto the electrode surface, and then **SS4** was used to hybridize to **SS3** to form ds-DNA for lysozyme determination.

3. Results and discussion

3.1. Electrochemical behavior of TPA at DNA-modified electrode

Fig. 1 illustrates the cyclic voltammetry (CV) of phosphate buffer saline containing 20 mM TPA at the bare, ss-DNA-modified, and ds-DNA-modified electrodes. An improved anodic currents were observed when ss-DNA or ds-DNA was attached onto the Au electrode. However, when the above as-prepared electrodes were scan the PBS without TPA, anodic peak was not observed. The factors effecting TPA oxidation were studied extensively because TPA is one of the most important co-reactants for ruthenium complex electrochemiluminesence (ECL). The hydrophobic electrode surface contributes to the improved TPA oxidation due to the preconcentration to TPA (Zu and Bard, 2000, 2001; Miao et al., 2002; Pittman and Miao, 2008; Li and Zu, 2004; Workman and Richter, 2000; Factor et al., 2001; Li et al., 2007c; Xu et al., 2007). The electrolytes with high pH (beyond 7.5) facilitate the deprotonation of co-reactants (Yin et al., 2008, 2009a). The anodic peak of TPA oxidation is significantly improved at ds-DNA-modified electrode (Fig. 1c against Fig. 1a) but DNA is hydrophilic and pH 7.5 electrolyte is used. In our previous work (Yin et al., 2009b), we proposed that the ds-DNA strand attached onto the electrode surface could preconcentrate the TPA through the electrostatic interaction between the anionic phosphate backbone and the protonated $TPAH^+$ (Scheme 1B) because of its acidity constant (K_a) of 10.4 (Kanoufi et al., 2001; Pastore et al., 2006). Moreover, the anionic phosphate backbone of DNA provided a alkaline microenvironment to facilitate the deprotonation of $TPAH^+$ (Yin et al., 2009b). The mechanism for the improved TPA oxidation at ds-DNA-modified electrode was proposed as follows (Zu and Bard, 2001; Miao et al., 2002; Kanoufi et al., 2001; Pastore et al., 2006):

$$TRAP^+ + DNA \rightarrow TRAP^+(DNA) \quad (1)$$

$$TRAP^+(DNA) \rightarrow TPA^{\bullet+} + H^+(DNA) + e^- \quad (2)$$

The previous works confirmed the different oxidation of TPA at ss-DNA- and ds-DNA-modified electrode (Pittman and Miao, 2008; Li et al., 2009). The TPA oxidation was improved due to the stable double-helix geometry of ds-DNA, which always presented the negatively charged phosphate backbone. However, the uncoiled ss-DNA is sufficient for the exposure of its bases (Huang et al., 2008; Li et al., 2009; Xiao et al., 2009). The intrinsic conductivity of ds-DNA strand also promoted the TPA oxidation over the ss-DNA (Pittman and Miao, 2008; Li et al., 2009). The above results provided the possibility of probing the dissociation of ds-DNA by the use of TPA oxidation.

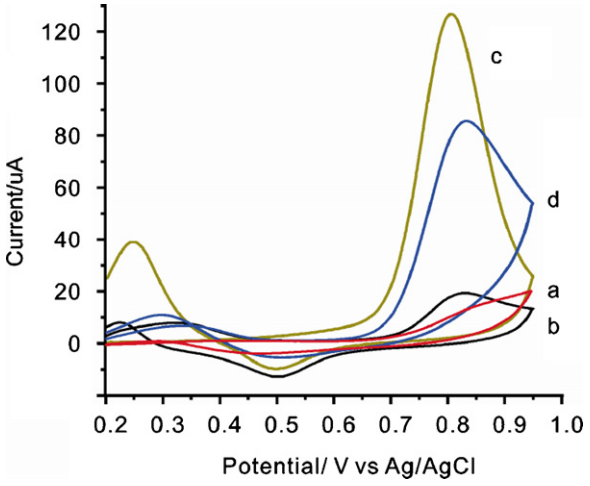

Fig. 1. Cyclic voltammograms in 0.1 M phosphate buffer solution (pH 7.5) containing 20 mM TPA by the use of (a) bare gold electrode; (b) the ss-DNA (**SS1**) modified gold electrode; (c) the gold electrode assembled with ds-DNA between the anti-aptamer ss-DNA and the immobilized **SS1**; (d) after the ds-DNA modified electrode in (c) reacted with 10 μL 100 pM lysozyme solution. Electrolyte: 0.1 M phosphate buffer solution (pH 7.5) containing 20 mM TPA.

3.2. Construction of the biosensor for the probing lysozyme

The electrochemical behavior of TPA was investigated to validate the possibility of the biosensor for probing lysozyme. As shown in Fig. 1, only little anodic peak was observed at the bare Au electrode at 0.89 V. When the ss-DNA was attached on the electrode surface, a slight improved oxidation current was observed with a negative shift of oxidation potential. The formation of ds-DNA achieved a high anodic current, similar to the previous reports (Yin et al., 2009b; Pittman and Miao, 2008; Li et al., 2009). Once lysozyme interacted to its aptamer and dissociated the ds-DNA, a decreased peak current was observed over that at ds-DNA-modified electrode (Fig. 1d against Fig. 1c). Comparing with the peak potential of ca. 0.80 V at ds-DNA-modified electrode, the currents reached their maximum at ca. 0.82 V by the use of the ss-DNA modified electrode or the ds-DNA-modified electrode being incubated with 100 pM lysozyme solution. This result validates the previous mechanism of TPA oxidation. Scheme 1 illustrates the procedure and the principle for lysozyme detection by the use of TPA oxidation to probe the intermolecular displacement.

As shown in Fig. S1, the CVs of the ds-DNA modified electrode at different scan rates were recorded in phosphate buffer solution (PBS) containing 20 mM TPA. The anodic peak corresponded to TPA oxidation. The anodic peak currents, *Ipc*, were directly proportional to the square root of the scan rates (inset of Fig. S1). The TPA oxidation was therefore controlled by diffusion through the DNA film (Pittman and Miao, 2008; Yin et al., 2009b).

We also test the possibility to immobilize anti-lysozyme aptamer, **SS3** and then to form ds-DNA with its complementary strand, **SS4**, for the detection of lysozyme. Although a decreased anodic current was observed, a relatively poor reproducibility was obtained possibly because the attached lysozyme interferes with the TPA oxidation. Additionally, it has previously been shown that a surface-bound aptamer did not retain its full solution-phase activity, presumably because the protein could not access the entire binding surface for steric reasons (Sadik et al., 2009; He et al., 2010). Hence, **SS1** and **SS2** were selectively used for the determination of lysozyme for the further work.

The isoelectic point of lysozyme is 11.0, so the positive nature of lysozyme provides the favorable for specific binding to its aptamer and the dissociation constant between lysozyme and its aptamer is <31 nM is achieved (Cheng et al., 2007; Pavlov et al., 2004). Therefore, various aptasensors were developed for the lysozyme determination using the displacement of lysozyme to the complementary strand of its aptamer. Teller et al. (2009) used the opening of hairpin due to the formation of analyte–aptamer complex to form DNAzyme, which was used to amplify sensing signal catalytically. Similarly, Peng et al. (2009) developed a faradic impedance spectroscopy aptasensor for lysozyme by the use of the change in the interfacial electron transfer resistance due to the displacement of complementary ss-DNA with lysozyme. Using DNAzyme–aptamer conjugates and displacement of analyte to the complementary strand of the aptamer, Li et al. (2007b) developed an amplified strategy for the analysis of small substrates and proteins. Upon incubation in lysozyme solution, significant changes in the charged DNA-modified electrode surface. Therefore, the net positive charge

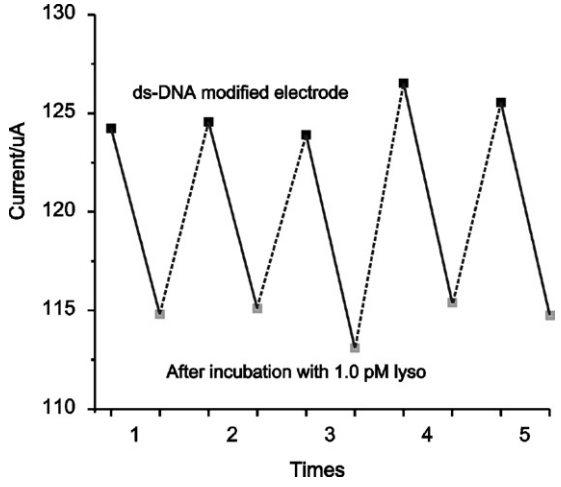

Fig. 2. The regeneration of ds-DNA modified electrode after being incubated with 1.0 pM lysozyme. The vertical axis is for the LSV peak currents recorded. Solid line is for the determination of 1.0 pM lysozyme and the dash line means the regeneration of the ds-DNA modified electrode. Electrolyte: 0.1 M phosphate buffer solution (pH 7.5) containing 20 mM TPA.

of lysozyme facilitates the desorption of TPA from the DNA strand besides the dissociation of ds-DNA. The decreased surface concentration of TPA achieves the decreased electrochemical signal after hybridization of lysozyme to its aptamer.

3.3. Regeneration of the biosensor for determination of lysozyme

The recognition of the biosensor is the procedure for the dissociation of ds-DNA strand. After each determination, a part of the ds-DNA attached onto the electrode is dissociated and becomes ss-DNA. The electrode after the determination is easily regenerated to form the ds-DNA-modified electrode through being incubated with the anti-lysozyme aptamer. The precision for the determination of lysozyme was tested within 5 days with the regenerated modified electrode. The day-to-day relative standard deviations (RSDs, $n = 5$) were 7.1% as shown in Fig. 2. Because the electrochemical signals were dependent on the DNA strands attached onto the electrode surface, the above results indicated that the gold–thiol bond existed still and the bases in the DNA strands were not oxidized with scanning potential of 0.95 V. Therefore, the biosensor allows reuse without losing in sensitivity.

In the previous works (Zu and Bard, 2000, 2001; Miao et al., 2002; Pittman and Miao, 2008; Li and Zu, 2004; Workman and Richter, 2000; Factor et al., 2001; Li et al., 2007c; Xu et al., 2007), TPA was used as co-reactant for ruthenium complex-based ECL. Although the ECL emission presented a high sensitivity, the high excited potential (ca. 1.10 V for the oxidation of $Ru(bpy)_3^{2+}$) may result in the oxidation damage to the biomolecules. For example, guanine was easily oxidized at about +1.0 V (Heng et al., 2005), lower than the oxidation potential of $Ru(bpy)_3^{2+}$. The oxidation of guanine and adenine at 1.030 V and 1.330 V was used as label-free biosensor (Rodriguez and Rivas, 2009). The results in Fig. 2 validated that the present biosensor based on TPA oxidation can be used as a solution to the oxidation damage (Heng et al., 2005; Rodriguez and Rivas, 2009), because the scanned potential is lower than that for the oxidation of the bases.

3.4. Specificity of the aptasensor

To evaluate the selectivity of the present electrochemical aptasensor for lysozyme, the effects of a series of competing species, including bovine hemoglobin (BHB), bovine serum albumin (BSA), ovalbuin (OB), arginine (arg), and histidine (his), were examined. As shown in Fig. 3, only lysozyme shows a much strong electrochemical response, while none of the other species (at 50 nM level) gave signals higher than one fifth of that produced by 1.0 pM lysozyme. The selectivity was determined to be at least 50,000-fold higher for lysozyme over the other species (1.0 pM lysozyme versus 50 nM interferents). The results validated that the present aptasensor responded to its target with high specificity because of no evidence for the binding of the interferents to the sensor. On the other hand, the direct analysis of diluted egg white sample with good recovery (as shown in Table S1) also proves its good selectivity, indicating that aptamer is a specific recognition element for its target.

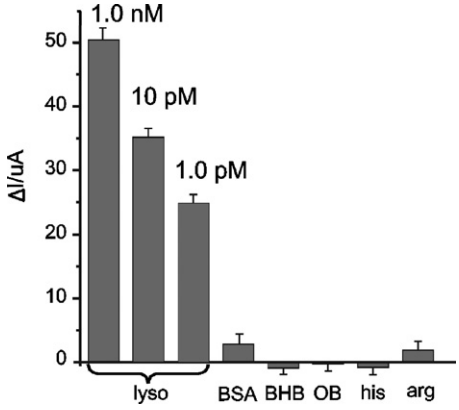

Fig. 3. Selectivity of the lysozyme aptasensor. The vertical axis is the changes of the currents before and after incubation with those species. All competing species were tested at 50 nM level. As a comparison, the response of 1.0 pM, 10 nM, 1.0 nM lysozyme is also presented. Electrolyte: 0.1 M phosphate buffer solution (pH 7.5) containing 20 mM TPA.

3.5. Analytical performace of the biosensor for lysozyme determination

Fig. 4 shows linear sweep volatammogram profiles for lysozyme with various concentrations. Along with the increase in the concentration of lysozyme, the decreased oxidation current of TPA was observed. Moreover, the anodic potential was shifted positively as shown in the arrow. It validated that the proportion of ss-DNA increased at the electrode surface, leading to the oxidation of TPA difficultly and a positive potential was needed. As shown

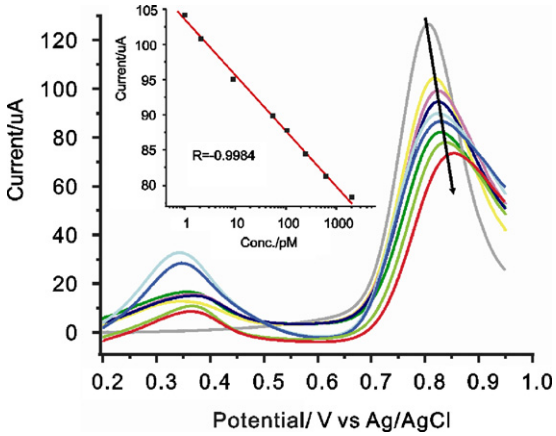

Fig. 4. Sensitivity of the lysozyme biosensor. Linear sweep voltammogram signal changes in the presence of varying concentrations of lysozyme. Inset: EC signal intensity for varying concentrations of lysozyme. Electrolyte: 0.1 M phosphate buffer solution (pH 7.5) containing 20 mM TPA.

in the inset of Fig. 4, the biosensor presents a linear range from 1.0 pM to 1.1 nM. The adsorbed capacity to $TPAH^+$ is possibly 3-fold of that $Ru(NH_3)_6^{3+}$ probe on the DNA strand under the same condition because of their difference in charge. This also contributed the sensitivity improvement of the present sensor. Table S1 shows the precision of the biosensor for real sample analysis with RSD ranged from 1.4 to 4.2%. Fig. 2 displays five typical replicate determinations within 5 days after the regeneration of the aptasensors. The day-to-day RSDs ($n = 5$) were 7.1%. All of the results indicate the good reproducibility of the proposed method.

A comparison of the detection limits of aptamer-based methods for lysozyme determination was achieved as shown in Table S2. The detection limits obtained in this work were lower than those in the most of the previous works. A label-free electrochemical aptasensor for lysozyme was developed with the detection limits of 18 nM (Rodriguez and Rivas, 2009). Bai et al. (2008) developed a method based on Ru[$(bpy)_2$(dcbpy)]NHS labelling for the ECL determination of lysozyme. In combination with Au nanoparticle amplification, the detection limits of 0.1 pM were obtained (Bai et al., 2008). However, the synthesis of both Au nanoparticle and probe molecule is needed. Therefore, a simple electrochemical biosensor is built to characterize the targets in the case concerning the hybridization and dissociation of ds-DNA with the high sensitivity and good reproducibility.

3.6. Application of the biosensor for lysozyme determination in real samples

We tested the capacity of the electrochemical sensor for the detection of lysozyme in a complex mixture with egg white as sample. With the high sensitivity of the improved TPA oxidation and the specificity of aptamer to lysozyme, the diluted egg white can be analyzed directly. 10 μL of the diluted egg white sample was dropped on the ds-DNA modified electrode and incubated at 36 °C for 1.5 h. After the electrode was washed thoroughly with 0.2 M PBS to reduce the non-specific binding, the electrochemical signals were recorded using PBS (pH 7.5) containing 20 mM TPA as electrolyte. As shown in Table S1, the calculated content of lysozyme in egg white sample was 2.62×10^{-4} M or 3.77 mg mL^{-1}, within the normal range of the previous reported values (Vidal et al., 2005). The recovery for the spike lysozyme with different concentrations in the diluted egg white sample ranged from 93 to 100% (Table S1). The precision for five replicate determinations at different concentration level ranged from 1.4 to 4.2% (RSDs, $n = 5$). The acceptable relative standard deviations and quantitative recoveries imply that the present aptasensor offers a promising feature for the analytical application in complex biological samples with a simple mode over the previous biosensors for lysozyme determination (Wang et al., 2009; Bai et al., 2008; Deng et al., 2009a; Peng et al., 2009; Du et al., 2009; Cho et al., 2006; Kawde et al., 2005).

4. Conclusions

Using different electrochemical behavior of TPA at ds-DNA- and ss-DNA-modified electrode, a simple but sensitive biosensor was successfully constructed for lysozyme in this work. The results indicated that the biosensor showed good stability, excellent selectivity, and high reproducibility. The electrostatic interaction between anionic phosphate backbone of DNA and $TPAH^+$ achieved the improved oxidation of TPA at ds-DNA modified electrode due to the preconcentration to TPA and the facilitating TPA oxidation. Because the signal is produced from the preconcentrated TPA at the electrode surface, a high sensitivity is achieved over the single site labelling strategy. This work opens a new field by probing the change between ds-DNA and ss-DNA at electrode surface for bioassay. With the various aptamers developed for proteins, the protocol can be easily extended to the analysis of other proteins (Willner and Zayats, 2007).

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.11.035.

References

Bai, J.G., Wei, H., Li, B.L., Song, L.H., Fang, L.Y., Lv, Z.Z., Zhou, W.H., Wang, E.K., 2008. Chem. Asia J. 3, 1935–1941.
Carter, M.T., Bard, A.J., 1990. Bioconjug. Chem. 1, 257–263.
Chakraborty, B., Jiang, Z.F., Li, Y.C., Yu, H.Z., 2009. J. Electroanal. Chem. 635, 75–82.
Cheng, A.K.H., Ge, B.X., Yu, H.Z., 2007. Anal. Chem. 79, 5158–5164.
Cho, E.J., Collett, J.R., Szafranska, A.E., Ellington, A.D., 2006. Anal. Chim. Acta 564, 82–90.
Deng, C.Y., Chen, J.H., Nie, L.H., Nie, Z., Yao, S.Z., 2009a. Anal. Chem. 81, 9972–9978.
Deng, C.Y., Chen, J.H., Nie, Z., Wang, M.D., Chu, X.C., Xiao, X.L.C., Lei, Y., Yao, S.Z., 2009b. Anal. Chem. 81, 739–745.
Du, Y., Chen, C.G., Li, B.L., Zhou, M., Wang, E.K., Dong, S.J., 2009. Biosens. Bioelectron. 25, 1902–1907.
Factor, B., Muegge, B., Workman, S., Bolton, E., Bos, J., Richter, M.M., 2001. Anal. Chem. 73, 4621–4624.
Gao, Z.Q., Tansil, N.C., 2009. Anal. Chim. Acta 636, 77–82.
He, J.L., Wu, Z.S., Zhou, H., Wang, H.Q., Jiang, J.H., Shen, G.L., Yu, R.Q., 2010. Anal. Chem. 82, 1358–1364.
He, P.L., Shen, L., Cao, Y.H., Li, D.F., 2007. Anal. Chem. 79, 8024–8029.
Heng, L.Y., Chou, A., Yu, J., Chen, Y., Gooding, J.J., 2005. Electrochem. Commun. 7, 1457–1462.
Huang, C.C., Chiu, S.H., Huang, Y.F., Chang, H.T., 2007. Anal. Chem. 79, 4798–4804.
Huang, Y.C., Ge, B.X., Sen, D., Yu, H.-Z., 2008. J. Am. Chem. Soc. 130, 8023–8029.
Jiang, Y.X., Fang, X.H., Bai, C.L., 2004. Anal. Chem. 76, 5230–5235.
Kanoufi, F., Zu, Y., Bard, A.J., 2001. J. Phys. Chem. B 105, 210–216.
Kawde, A.N., Rodriguez, M.C., Lee, T.M.H., Wang, J., 2005. Electrochem. Commun. 7, 537–540.
Kerman, K., Saito, L.M., Yamamura, S., Takamura, Y., Tamiya, E., 2008. Trends Anal. Chem. 27, 585–592.
Li, B.L., Wei, H., Dong, S.J., 2007a. Chem. Commun., 73–75.
Li, D., Shylabovasky, B., Elbaz, J., Willner, I., 2007b. J. Am. Chem. Soc. 129, 5084–5085.
Li, F., Zu, Y.B., 2004. Anal. Chem. 76, 1768–1772.
Li, J., Huang, M.H., Liu, X.Q., Wei, H., Xu, Y.H., Xu, G.B., Wang, E.K., 2007c. Analyst 132, 687–691.
Li, L., Li, B.X., Qi, Y.Y., Jin, Y., 2009. Anal. Bioanal. Chem. 393, 2051–2057.
Miao, W.J., Choi, J.-P., Bard, A.J., 2002. J. Am. Chem. Soc. 124, 14478–14485.
Nutiu, R., Li, Y., 2003. J. Am. Chem. Soc. 125, 4771.
Odenthal, K.J., Gooding, J.J., 2007. Analyst 132, 603–610.
Pastore, P., Badocco, D., Zanon, F., 2006. Electrochim. Acta 51, 5394–5401.
Pavlov, V., Xiao, Y., Shylabovasky, B., Willner, I., 2004. J. Am. Chem. Soc. 126, 11768–11769.
Peng, Y.G., Zhang, D.D., Li, Y.H., Qi, L., Gao, Q., Zhang, C.X., 2009. Biosens. Bioelectron. 78, 94–99.
Pittman, T.L., Miao, W.J., 2008. J. Phys. Chem. C 112, 16999–17004.
Rodriguez, M.C., Rivas, G.A., 2009. Talanta 78, 212–216.
Sadik, O.M., Aluoch, A.O., Zhou, A., 2009. Biosens. Bioelectron. 24, 2749–2765.
Shen, L., Chen, Z., Li, Y.H., Jing, P., Xie, S.B., He, S.L., He, P.L., Shao, Y.H., 2007. Chem. Commun., 2169–2171.
Tansil, N.C., Xie, H., Xie, F., Gao, Z.Q., 2005. Anal. Chem. 77, 126–134.
Teller, C., Shimron, S., Willner, I., 2009. Anal. Chem. 81, 9114–9119.
Vidal, M.L., Gautron, J., Nys, Y., 2005. J. Agric. Food Chem. 53, 2379–2385.
Wang, J.L., Wang, F., Dong, S.J., 2009. J. Electroanal. Chem. 626, 1–5.
Wang, J., Jiang, Y.X., Zhou, C.S., Fang, X.H., 2005. Anal. Chem. 77, 3542–3546.
Willner, I., Zayats, M., 2007. Angew. Chem. Int. Ed. 46, 6408–6418.
Workman, S., Richter, M.M., 2000. Anal. Chem. 72, 5556–5561.
Xiao, Y., Lubin, A.A., Heeger, A.J., Plaxco, K.W., 2005. Angew. Chem. Int. Ed. 44, 5456–5459.
Xiao, Y., Uzawa, T., White, R.J., DeMartini, D.K., Plaxco, W., 2009. Electroanalysis 21, 1267–1271.
Xu, Y.H., Gao, Y., Li, T., Du, Y., Li, J., Wang, E.K., 2007. Adv. Funct. Mater. 17, 1003–1009.
Xu, Y., Yang, L., Ye, X.Y., He, P.G., Fang, Y.Z., 2006. Electroanalysis 18, 1449–1456.

Yin, X.B., Sha, B.B., He, X.W., 2009a. Sci. China Ser. B-Chem. 52, 1394–1401.
Yin, X.B., Sha, B.B., Zhang, X.H., He, X.W., Xie, H., 2008. Electroanalysis 20, 1085–1091.
Yin, X.B., Xin, Y.Y., Zhao, Y., 2009b. Anal. Chem. 81, 9299–9305.
Zhang, J., Song, S., Zhang, L., Wang, L., Wu, H., Pan, D., Fan, C., 2006. J. Am. Chem. Soc. 128, 8575.
Zu, Y.B., Bard, A.J., 2000. Anal. Chem. 72, 3223–3232.
Zu, Y.B., Bard, A.J., 2001. Anal. Chem. 73, 3960–3964.