Analytica Chimica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Fabricating a reversible and regenerable electrochemical biosensor for quantitative detection of antibody by using "triplex-stem" DNA molecular switch

Wei Wei, Linqun Zhang, Qiangwei Ni, Yuepu Pu, Lihong Yin, Songqin Liu *

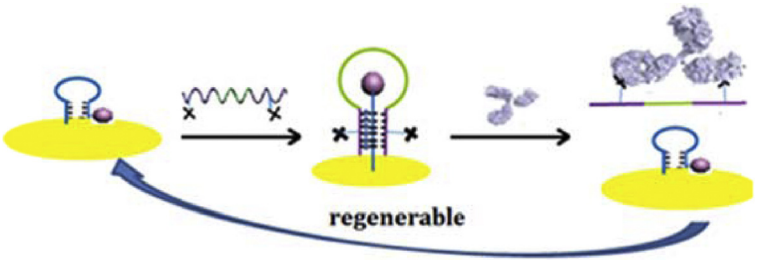
Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, Jiangsu, PR China

HIGHLIGHTS

- This biosensor overcomes the limitations of the ELISA or sandwich-type assays.
- The sensors work well when challenged with complex, contaminant-ridden samples.
- Stability of the "triplex-stem" DNA is adjusted conveniently and efficiently by Ag^+.
- The DNA switch architecture reduces the cost and complexity of the synthesis.
- The biosensor shows excellent reproducibility by simple heating in water bath.

GRAPHICAL ABSTRACT

Fabricating a reversible and regenerable electrochemical biosensor for quantitative detection of antibody by using "triplex-stem" DNA molecular switch.



ARTICLE INFO

Article history:
Received 15 May 2014
Received in revised form 2 July 2014
Accepted 15 July 2014
Available online xxx

Keywords:
Electrochemical biosensor
Antibody detection
Molecular switch
DNA
Digoxin
Silver ion

ABSTRACT

A reversible and regenerable electrochemical biosensor is fabricated for quantitative detection of antibody based on "triplex-stem" molecular switches. A hairpin-shaped oligonucleotide (hairpin DNA) labeled with ferrocene (Fc) at the 3′-end is fixed on the gold electrode serving as a signal transduction probe. Its hairpin structure leads Fc close to the surface of gold electrode and produces a strong current signal (on-state). A single-strand oligonucleotide modified with two digoxin molecules on the two arm segments (capture DNA) interact with hairpin DNA with the help of Ag^+ ions. The "triplex-stem" DNA forms, which separates Fc from the electrode and reduces the electrochemical signal (off-state). Binding of digoxin antibody to digoxin releases capture DNA from the hairpin DNA, creating an effective "off-on" current signal switch. The stability of the "triplex-stem" structure of hairpin/capture DNA is critical to the signal switch and the sensitivity of the method, which can be adjusted conveniently and efficiently by changing Ag^+ concentrations. Based on the "off-on" current signal switch, this biosensor is used to detect digoxin antibody sensitively in blood serum. The linear range is 1.0–500 pg with a correlation coefficient of 0.996, and the detection limit is 0.4 pg. Also, this biosensor shows excellent reversibility and reproducibility, which are significant requirements for practical biosensor applications.

Abbreviations: Fc, ferrocene; UV, ultra violet; EIS, electrochemical impedance spectroscopy; DPV, differential pulse voltammetry; PBS, phosphate buffer solution; ELISA, enzyme linked immunosorbent assay; RSD, relative standard derivation.

* Corresponding author. Tel.: +86 25 52090613; fax: +86 25 52090618.
E-mail address: Liusq@seu.edu.cn (S. Liu).

http://dx.doi.org/10.1016/j.aca.2014.07.015
0003-2670/

1. Introduction

Antibody is an indicator of observation curative effect and prognosis, inoculation effect of vaccine in some disease. Detection antibody is significant for the diagnosis of many disease states, including epidemiology diseases, autoimmune diseases, and allergies [1]. The rapid, low-cost detection methods would exert great impacts on human's health by taking more frequent testing, narrowing the delay between diagnosis and treatment.

In reported papers, variety of analytical techniques has been developed for detecting antibody in blood serum, saliva, and other body fluids. Traditional methods include ELISA [2] and other heterogeneous, sandwich-type assays [3,4]. Selected haptenic groups are immobilized on a surface, followed by addition of a sample potentially containing antibodies, which bind to the hapten. The secondary antibody that specific for the analyte antibody class (e.g., IgG) and labeled with a tag such as a fluorescent molecule or an enzyme are prepared to detect these immobilized antibodies. Requiring a secondary antibody increases analytical and incubation steps and thus increases both the analysis time and the risk of nonspecific binding, leading to false positives.

To overcome the limitations of the ELISA method or sandwich-type assays, some efforts have been made. Sambashiva groups utilized the antibody-induced disruption of an intramolecular interaction between TEM1 β-lactamase and its inhibitor protein BLIP to indirectly detect specific antibody [5]. Ober have developed a sensor platform based on the antibody-catalyzed water oxidation pathway that takes advantage of the intrinsic capacity of single antibodies to catalyze the production of hydrogen peroxide from water in the presence of singlet oxygen, which can be generated by a photosensitizer [6]. Guo reengineered an epithelial cell adhesion molecule (EpCAM) peptide into the C-terminal of nanopore as a probe to specifically detect EpCAM antibody (Ab) in nanomolar concentration at the single molecule level. The signal of EpCAM antibody can be discriminated from the background events in the presence of nonspecific antibody or serum [7]. Microscale thermophoresis has been demonstrated to direct measurements of antibody affinity and concentration in 50% serum by Braun groups [8]. Pentacene-based organic thin-film transistors have been used to create highly sensitive, real-time electronic sensors for selective antibody detection [9].

Due to the stability and associativity of DNA, the electrochemical DNA biosensor was widely used to detect specific DNA [10–13] and RNA sequences [14–16], proteins [17,18], molecules [19], cells [20,21], inorganic ions [22]. Because all of the sensing components in the electrochemical DNA platform are covalently attached to the electrode, the approach requires neither exogenous reagents nor labeling of the target [23]. In addition, because their signaling is linked to specific, binding-induced changes in the dynamics of the probe DNA (rather than changes in adsorbed mass, charge, etc.), these sensors work well when challenged with complex, contaminant-ridden samples such as blood serum, soil extracts, and food stuffs [24].

Here, we designed an electrochemical DNA biosensor by taking advantage of the occurrence of two antigen-binding sites on each antibody [25] and "triplex-stem" DNA molecular switch [26]. The hairpin DNA brings Fc close to the surface of gold electrode and produces a strong electrochemical signal via the formation of a stem-loop structure (on-state). Capture DNA can trigger the DNA molecular switch by forming "triplex-stem" structure with hairpin DNA in the presence of Ag^+. As a result, the hairpin DNA was open, causing the decreased electrochemical current (off-state). The combination of digoxin with digoxin antibody releases capture DNA, as a result, the triplex-stem structures are destroyed and the hairpin DNA restores its "duplex-stem" cyclic structure, creating an effective "off–on" current signal switch. Based on the "off–on" current signal switch, this biosensor is used to detect digoxin antibody sensitively in blood serum. Compared to the reported switch architecture that require the synthesis of a tetra-modified DNA strand containing two antigens and the anchor moiety and redox label (or the fluorophore and quencher in the case of the optical switch), this DNA switch architecture reduces the cost and complexity of the synthesis by labeling only two antigens on the capture DNA. Compared to similar reported literature [27] where the moderate stability of the "triplex-stem" DNA, which is very critical for the signal switch and the sensitivity of the method, was adjusted by changing the number of the base in the "triplex-stem", it is very convenient and efficient in this method by adjusting the Ag^+ concentration. Also, this biosensor shows excellent reversibility and reproducibility, which are significant requirements for practical biosensor applications.

2. Experimental

2.1. Reagents

The digoxin antibody, hairpin DNA (SH-GCATA-TAGGGTGGGTTTTTTCTATAAT-Fc (5′-3′), the matched bases are underlined) and capture DNA (CCC/iDigdT/CCCTTTTTTTTTTTTTTTTTTCCC/iDigdT/CCC(5′-3′)) were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). The primitive digoxin antibody sample is stored in −20 °C. The primitive DNA sample was centrifuged at 5000 rpm for 5 min and then dissolved with 10 mM pH 7.4 PBS. Then the solution was deposited at 0–4 °C for storage.

2.2. Apparatus

The electrochemical experiments were performed with a model CHI 830C electrochemical workstation (Shanghai Chenhua Equipments, China). The three-electrode system was consisted of a KCl saturated calomel electrode as the reference, a platinum electrode as the counter electrode, and a gold electrode (2 mm diameter) as the working electrode. The instrument used to heat the sample was DF-101S heat-gathering magnetic heating stirrer (Gongyi Kerui Instrument Co., Ltd.).

CD spectra and UV melting experiment were measured at room temperature on a Chirascan™-plus circular dichroism spectrometer (Applied Photophysics Ltd., England).

2.3. Procedures

2.3.1. Immobilization of hairpin DNA on gold electrode

Gold electrodes (99.99% polycrystalline, 2 mm diameter, CH Instrument Inc.) were treated with piranha solution (H_2O_2/H_2SO_4 1:3 in volume), polished on microcloth (Buehler) with 0.3 μm gamma alumina suspension (CH Instrument Inc.) for 2 min, and again rinsed with ultrapure water and ethanol followed by drying under mild nitrogen flow. The pretreated gold electrode was immersed in 10 mM pH 7.4 PBS containing 1 μM hairpin DNA and 1 M NaCl for 16 h at room temperature. Then, the SH— modified hairpin DNA was assembled on the electrode by Au—S bonds. Adsorbed DNA was removed by rinsing the electrode. Finally, the modified electrode was dried under a stream of nitrogen.

2.3.2. Formation of "triplex-stem" DNA on the electrode

The modified electrode was then placed in 10 mM pH 7.4 phosphate buffer containing 200 mM $NaNO_3$, 2.5 mM $C_4H_6O_4Mg{\cdot}4H_2O$, 10 μM $AgNO_3$ and 1.0 μM capture DNA for 2 h at room temperature. Then the electrode was washed three times with phosphate buffer solution and blow-dried with nitrogen.

Please cite this article in press as: W. Wei, et al., Fabricating a reversible and regenerable electrochemical biosensor for quantitative detection of antibody by using "triplex-stem" DNA molecular switch, Anal. Chim. Acta (2014), http://dx.doi.org/10.1016/j.aca.2014.07.015

Then, the "triplex-stem" DNA formed due to the interaction between the hairpin DNA and capture DNA.

2.3.3. Electrochemical detection of digoxin antibody

The above modified gold electrode was immersed in the digoxin antibody diluents for 45 min incubation. The electrochemical measurements were performed in 10 mM pH 7.4 PBS containing 0.1 M $KClO_4$ at room temperature. The DPV curves were background-subtracted using ORIGIN 8.5 (Microcal Software, Northampton, MA) through extrapolation to the baseline in the regions far from the peaks. The electrochemical impedance spectroscopy (EIS) experiments were measured in 5 mM Fe $(CN)_6^{3-/4-}$ solution containing 0.1 M KCl with the frequency range from 1 Hz to 10 kHz. The used hairpin DNA modified electrode is heated to 70 °C in water bath to remove unreleased capture DNA for next detection.

3. Results and discussion

3.1. Scheme of the DNA electrochemical biosensor

The design of this approach is illustrated in Scheme 1. Hairpin DNA labeled with ferrocene (Fc) at its 3′-end and —SH at its 5′-end is fixed on the gold electrode by Au—S bonds. Its hairpin structure put Fc close to the gold electrode. As a result, strong current by DPV method was obtained (curve a, Fig. 1). Single-strand oligonucleotide that modified with two digoxin molecules on its two arm segments are designed as capture DNA. One arm segment of the capture DNA with six cytosine bases hybridizes with G bases in the hairpin DNA. At pH 7.4, "triplex-stem" structure formed in the presence of Ag^+ due to the structure of CG.CAg^+. In this state, Fc was physically separated from the electrode surface, resulting in sharply reduced electrochemical signal (curve b, Fig. 1). Upon two antigen-binding sites binding to each digoxin antibody, capture DNA is released from the hairpin DNA. As a result, "triplex-stem" structures are destroyed and the hairpin DNA restores its "duplex-stem" cyclic structure. Fc was brought into proximity to the gold electrode surface again, recovering its current (curve c, Fig. 1). The recovery efficiency of the current is dependent on the concentration of digoxin antibody.

3.2. Optimal conditions for the formation of "triplex-stem" DNA

The stability of the "triplex-stem" DNA was strongly influenced by pH, Ag^+ concentration and temperature. At acidic conditions, the C bases in another segment of capture DNA are protonated at their N3 positions ($pK_a = 4.5$) and interact with G bases in the hairpin DNA that have already interacted with C bases in capture DNA by Hoogsteen hydrogen bonds to form triplex conformation "CG.C$^+$" (Fig. 2A). However, at pH 7.4 these unpaired C bases are deprotonated. As a result, "CG.C$^+$" triplex conformations are not stable. The hairpin DNA is still "on" state and the current (curve d, Fig. 1) are strong as that for the free hairpin DNA (curve a, Fig. 1). In the presence of Ag^+, an "triplex-stem" structures forms due to the specific bridge of C—Ag^+—C (Fig. 2B) [26]. In this situation, the hairpin DNA are "off" state and the current are decreased sharply (curve b, Fig. 1).

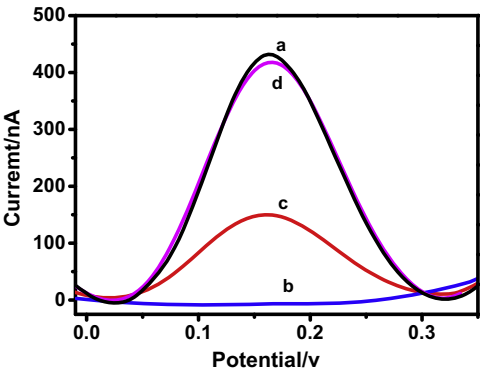
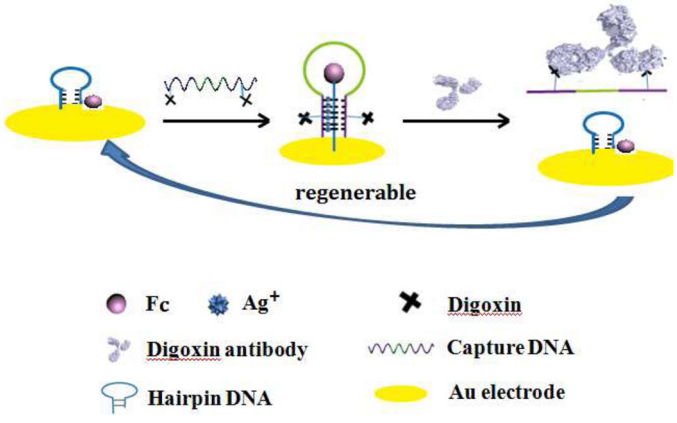


Fig. 1. DPV responses of the biosensor. The gold electrode was modified with (a) hairpin DNA; (b) hairpin/capture DNA in the presence of Ag^+; (c) the presence of digoxin antibody in the (b); (d) hairpin/capture DNA in the absence of Ag^+.

Thus, the stability of the "triplex-stem" structure is dependent strongly on the concentrations of Ag^+. Fig. 3a shows the effect of the concentration of Ag^+ on the decreased currents in the presence of various concentrations of Ag^+ (I_1) compared to that without Ag^+ (I_0). The decreased ratio of currents $((I_0 - I_1)I_0)$ increases with its increasing concentration and reaches a plateau at 10 μM Ag^+, reflecting that the stablest "triplex-stem" structure forms in the presence of 10 μM Ag^+. After incubation the gold electrode that modified with "triplex-stem" DNA with digoxin antibody for 45 min, the electrochemical signal recovered apparently and the signal ratio $((I_2 - I_1)I_0)$ increased with the increasing Ag^+ from 0 to 10 μM. Further increased Ag^+ led to decreased signal recovery (Fig. 3b) because too stable "triplex-stem" DNA formed and in this case, more digoxin antibody needed to separate capture DNA from hairpin DNA, which is disadvantage to the sensitivity of the method. Compared to similar reported literature where the moderate stability of the triplex-stem DNA was adjusted by changing the number of the base in the "triplex-stem" [27], this method is very convenient and efficient by adjusting the Ag^+ concentration. Thus, 10 μM Ag^+ are optimum conditions for the stability and sensitivity of the method.

Temperature is another important factor for the stability of these triplex structures. As shown in Fig. 4A, the current decrease efficiency increased when the hybridization temperature increased from 4 °C to 25 °C and decreased with further increased temperature (curve a), which indicated that these "triplex-stem" structure are stablest at 25 °C. This is coincidence well with the UV melting experimental results (Fig. 4B). Melting experiments showed that the "triplex-stem" DNA experienced two melting procedures with the increasing temperature and they are stable at 25 °C. The two melting procedures correspond to triplex–duplex (t–d) transitions (at 40 °C) and duplex-coil (d–c) transitions (at 62.5 °C), respectively [28]. After incubation with digoxin antibody, the current recovery efficiency increased from 4 °C to 25 °C and tended to reduce with further heating to 55 °C (curve b, Fig. 4A). High current decrease efficiency induced by capture DNA and recovery efficiency induced by digoxin antibody are advantage to increase the detection



Scheme 1. Scheme of the detection of digoxin antibody by using "triplex-stem" DNA molecular switch.

Please cite this article in press as: W. Wei, et al., Fabricating a reversible and regenerable electrochemical biosensor for quantitative detection of antibody by using "triplex-stem" DNA molecular switch, Anal. Chim. Acta (2014), http://dx.doi.org/10.1016/j.aca.2014.07.015

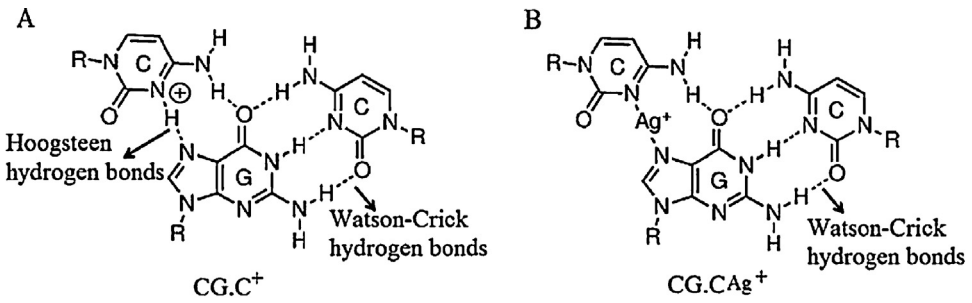


Fig. 2. Mechanism of the formation of "triplex-stem" DNA structure based on pH and Ag^+. (A) At acidic conditions by hoogsteen hydrogen bonds. (B) At pH 7.4, with the help of Ag^+.

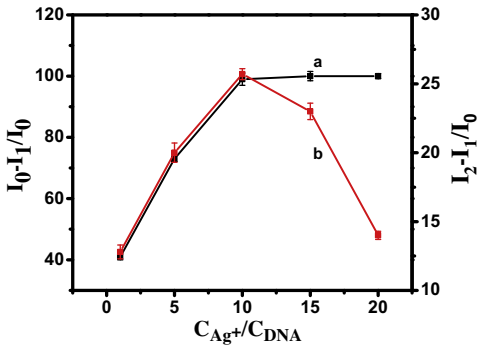


Fig. 3. The effect of Ag^+ concentration on the (a) current decrease efficiency when capture DNA hybridized with hairpin DNA; (b) current recovery efficiency when digoxin antibody was incubated with "triplex-stem" DNA. Conditions: 10 mM PBS (pH 7.4, 200 mM $NaNO_3$, 2.5 mM $C_4H_6O_4Mg{\cdot}4H_2O$), DPV, pulse amplitude, 50 mV; pulse period, 0.2 s. Room temperature.

sensitivity. Thus, all the experiments are operated at room temperature (25 °C).

3.3. Characterization of the formation of "triplex-stem" DNA

In order to prove the formation of "triplex-stem" DNA, CD spectra of hairpin DNA, capture DNA and their mixture in the presence of (Fig. 5A) and absence of Ag^+ (Fig. 5B) were recorded. In the absence of Ag^+, compared with the spectra of hairpin DNA (a) and capture DNA (b), no new characteristic peak appeared in the spectra of their mixture (c) except that their ellipticity increased, which indicated that no new structure formed (Fig. 5A). While in the presence of Ag^+, a new characteristic peak around 285 nm appeared in the spectra of their mixture (c) compared with the spectra of free hairpin DNA (a) and capture DNA (b) (Fig. 5B), which proves the formation of "triplex-stem" DNA [29]. The melting curves in Fig. 5C showed the change of the CD spectra of "triplex-stem" DNA with the increase of temperature. From 20 °C to 90 °C, the ellipticity at 200 nm decrease gradually and the negative peak at 260 nm and the positive peak at 285 nm shift to short wavelength gradually companied with slight change of their ellipticity, which indicate that their "triplex-stem" structure changes with the increase of temperature.

3.4. EIS measurements

The EIS experiments are used to prove each treatment for the electrode (Fig. 5). The equivalent circuit R(Q(RW)) is chosen to fit the EIS data (shown as an inset in Fig. 6). The components in the equivalent circuit include the solution resistance Rs, the charge-transfer resistance Rct constant phase element Q related to double-layer capacitance and Warburg impedance W [30]. The bare gold electrode behaves as an ideal conductor and the impedance spectrum give a linear plot (a). The resistance is about 130 $\Omega\,cm^{-2}$ obtained by computer fitting of the experimental spectrum using the equivalent circuit. After modified with SH— labeled hairpin DNA, Faraday impedance of the gold electrode increases to 3352 $\Omega\,cm^{-2}$ (b) because the negative charge on the capture DNA 2 make electron transfer of ferricyanide/ferrocyanide probes become difficult due to the electrostatic repulsion. Digoxin antigen is fitted on the electrode surface when capture DNA hybridized with hairpin DNA, as a result, the resistance increase to 8539 $\Omega\,cm^{-2}$ because of the insulating property of the protein layer (c). After the incubation of gold electrode with digoxin antibody, the resistance decrease to 7154 $\Omega\,cm^{-2}$, which proved that specific binding of antibody and antigen destroyed the "triplex-stem" conformation, releasing

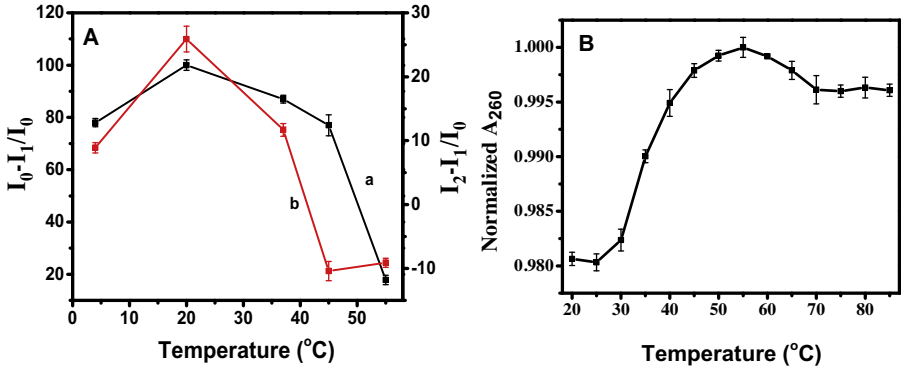


Fig. 4. (A) The effect of temperature on (a) current decrease efficiency induced by the hybridization of capture DNA with hairpin DNA; (b) current recovery efficiency when digoxin antibody was incubated with "triplex-stem" DNA. (B) The UV melting curves of the "triplex-stem" DNA at 260 nm. The data were collected at a heating rate of 5 °C min^{-1} and the range is 20–90 °C. Conditions are as the same as that in Fig. 3.

Please cite this article in press as: W. Wei, et al., Fabricating a reversible and regenerable electrochemical biosensor for quantitative detection of antibody by using "triplex-stem" DNA molecular switch, Anal. Chim. Acta (2014), http://dx.doi.org/10.1016/j.aca.2014.07.015

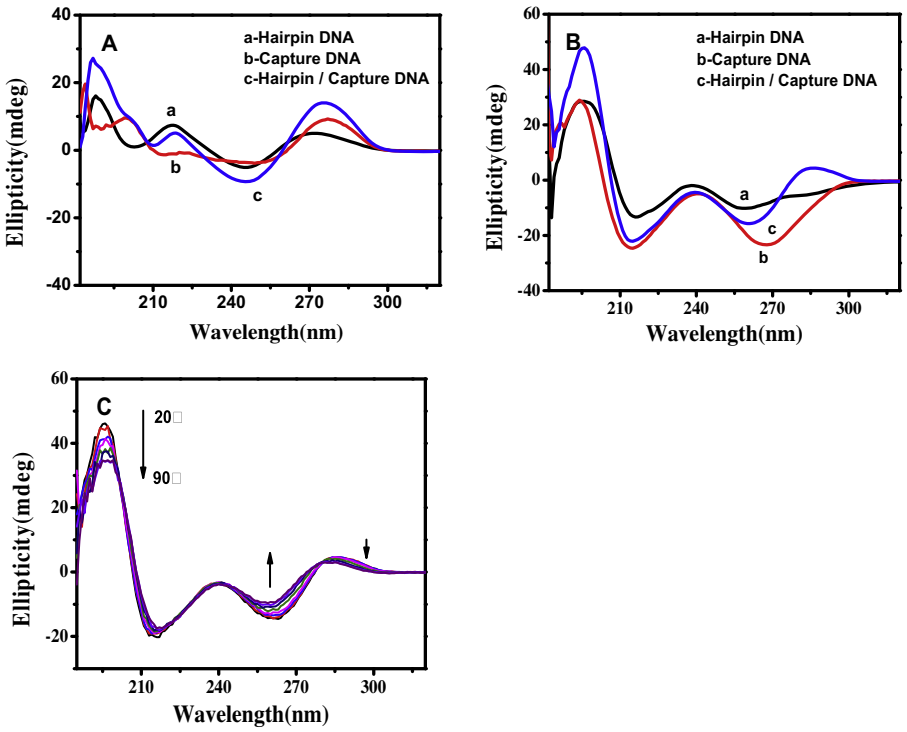


Fig. 5. CD spectra of hairpin DNA, capture DNA and hairpin/capture DNA in the presence of (A) and absence of (B) Ag^{+}, and the CD melting curves of the "triplex-stem" DNA (C).

the capture DNA and recovering the "duplex-stem" cyclic structure of hairpin DNA. These EIS data provide useful information to support the surface change of the electrode during the modification and the detection process.

3.5. Assay performance of the electrochemical biosensor

3.5.1. Calibration curve for the detection of digoxin antibody

The DPV responses of the sensor to various concentrations of digoxin antibody are shown in Fig. 7. Dynamically increased peak currents are observed in the presence of increasing concentration of digoxin antiboy from 1.0 pg mL^{-1} to 100 μg mL^{-1}. A linear response versus the logarithm of digoxin antibody concentration is obtained in the range of 1.0–500 pg mL^{-1}, with a correlation coefficient of 0.996 (inset in Fig. 7). The detection limit is 0.4 pg mL^{-1} (5.8 fM) obtained in terms of three times deviation of blank sample, which is far lower than reported detection limit of 0.3 nM [24].

3.5.2. Selectivity of the biosensor

100 ng mL^{-1} of anti-Goat, anti-CEA, anti-TNF, anti-IgG, and anti-HCG were used to investigate the selectivity of the biosensor (Fig. 8). The current recovery efficiency for anti-TNF was about 15% of that for 1.0 ng mL^{-1} digoxin antibody. For all other antibody the signal ratio was lower than 10%. This result indicated that the biosensor was able to discriminate digoxin antibody from other types of antibody with high specificity.

3.5.3. Detection of digoxin antibody in blood serum

Digoxin antibody in blood serum has been detected by this method. Three various concentrations (1.0 ng, 250 pg, and 10 pg) of

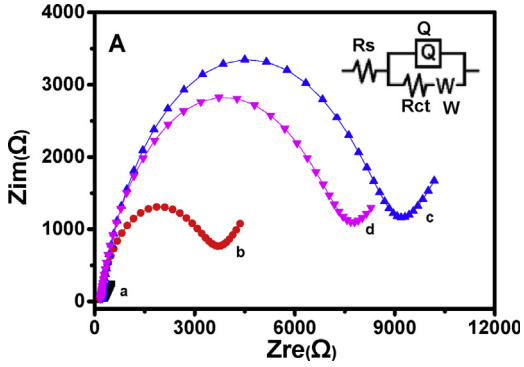


Fig. 6. EIS of (a) bare; (b) hairpin DNA modified; (c) capture/hairpin DNA modified electrode; (d) incubation of (c) with digoxin antibody. Conditions: 10 mM pH 7.4 PBS containing 5 mM Fe(CN) $6^{3-}/^{4-}$; frequency range, 1–10 kHz; ac amplitude, 10 mV.

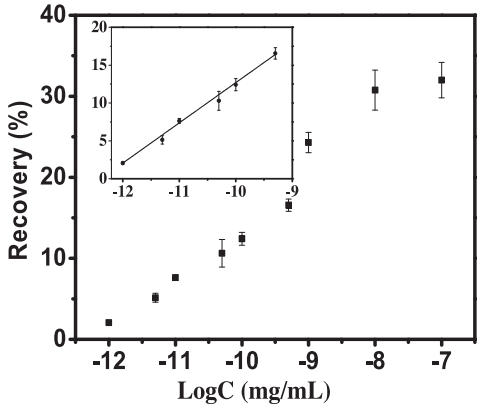


Fig. 7. The recovery efficiency of current of the DNA biosensor at various concentrations of digoxin antibody from 1.0 pg mL^{-1} to 100 ng mL^{-1}. Inset: calibration curve for digoxin antibody.

Please cite this article in press as: W. Wei, et al., Fabricating a reversible and regenerable electrochemical biosensor for quantitative detection of antibody by using "triplex-stem" DNA molecular switch, Anal. Chim. Acta (2014), http://dx.doi.org/10.1016/j.aca.2014.07.015

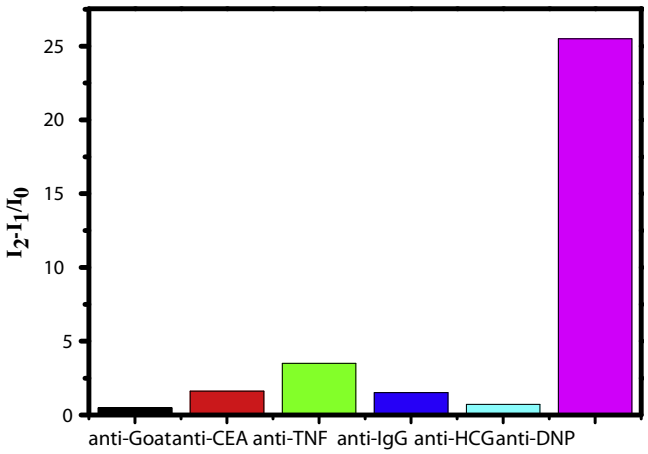


Fig. 8. Specificity of the biosensor for detection of digoxin antibody. Conditions: 100 ng mL^{-1} of each control type of antibody and 1.0 ng mL^{-1} of digoxin antibody.

Table 1
Detection of digoxin antibody in blank blood serum samples.

Sample	Added	Found	Recovery (%)	RSD (%, n = 9)
1	1.0 ng	1 ng	100	3.75
2	1.0 ng	0.95 ng	95	
3	1.0 ng	0.93 ng	93	
4	250 pg	263 pg	105	6.8
5	250 pg	229 pg	91.6	
6	250 pg	245 pg	98	
7	10 pg	10.2 pg	102	13.3
8	10 pg	9.77 pg	97.7	
9	10 pg	10.5 pg	105	

digoxin antibody were added in blank blood serum. Recovery test results are shown in Table 1. Data showed that recoveries for the spiked samples ranged from 91.6% to 105% and relative standard derivation (RSD) was from 3.75% to 13.3%, which indicates that the method possess high accuracy and good repeativity.

4. Conclusion

A reversible and regenerable DNA biosensor was constructed by fixing Fc labeled oligonucleotide on the gold electrode as a signal transduction probe. A "triplex-stem" structure formed due to the interaction between the hairpin DNA and the capture DNA, whose stability can be adjusted conveniently and efficiently by adjusting the concentration of Ag^+. Digoxin antibody can combine with digoxin molecules that labeled on the capture DNA and release it from the hairpin DNA. As a result, the "duplex-stem" cyclic structure of the hairpin DNA restored and the current changed from an "off-state" to an "on-state". Based on this "off–on" current signal switch, this biosensor is used to detect digoxin antibody sensitively in blood serum. The linear range is 1.0–500 pg with a correlation coefficient of 0.996, and the detection limit is 0.4 pg. The biosensor shows excellent stability and sensitivity, which are significant requirements for practical biosensor applications.

Acknowledgments

The project is supported by the National Natural Science Foundation of China (Grant No. 21205014), the Natural Science Foundation of Jiangsu province (BK2012734), the Social Development Project of Jiangsu (BE2012731), and the Social Development Project of Suzhou (ZXY2012027).

References

[1] R.A. Manz, A.E. Hauser, F. Hiepe, A. Radbruch, Maintenance of serum antibody levels, Annu. Rev. Immunol. 23 (2005) 367–386.
[2] A.F.C. Nassar, S. Miyashiro, F. Gregori, R.M. Piatti, G.T. Daniel, L. Gregory, Standardization of an enzyme-linked immunosorbent assay (ELISA) for detection of antibodies anti-corynebacterium pseudotuberculosis in sheep, Small Rumin. Res. 116 (2014) 229–232.
[3] Q. Yu, H. Yang, F. Guan, Y.M. Feng, X.L. Yang, Y.H. Zhu, Detection of IgG in sera of patients with schistosomiasis japonica by developing magnetic affinity enzyme-linked immunoassay based on recombinant 14-3-3 Protein, Trans. R. Soc. Trop. Med. Hyg. 108 (2014) 37–41.
[4] S.S. Nielsen, N. Toft, Bulk tank milk elisa for detection of antibodies to mycobacterium avium subsp paratuberculosis: correlation between repeated tests and within-herd antibody-prevalence, Prev. Vet. Med. 113 (2014) 96–102.
[5] S. Banala, S.J.A. Aper, W. Schalk, M. Merkx, Switchable reporter enzymes based on mutually exclusive domain interactions allow antibody detection directly in solution, ACS Chem. Biol. 8 (2013) 2127–2132.
[6] M.E. Welch, N.L. Ritzert, H. Chen, N.L. Smith, M.E. Tague, Y. Xu, B.A. Baird, H.D. Abruña, C.K. Ober, Generalized platform for antibody detection using the antibody catalyzed water oxidation pathway, J. Am. Chem. Soc. 136 (2014) 1879–1883.
[7] S.Y. Wang, F. Haque, P.G. Rychahou, B.M. Evers, P.X. Guo, Engineered nanopore of Phi29 DNA-packaging motor for real-time detection of single colon cancer specific antibody in serum, ACS Nano 7 (2013) 9814–9822.
[8] S. Lippok, S.A.I. Seidel, S. Duhr, K. Uhland, H.P. Holthoff, D. Jenne, D. Braun, Direct detection of antibody concentration and affinity in human serum using microscale thermophoresis, Anal. Chem. 84 (2012) 3523–3530.
[9] H.U. Khan, J. Jang, J. Kim, W. Knoll, In situ antibody detection and charge discrimination using aqueous stable pentacene transistor biosensors, J. Am. Chem. Soc. 133 (2011) 2170–2176.
[10] A.A. Lubin, K.W. Plaxco, Folding-based electrochemical biosensors: the case for responsive nucleic acid architectures, Acc. Chem. Res. 43 (2010) 496–505.
[11] D. Wu, B.C. Yin, B.C. Ye, A label-free electrochemical DNA sensor based on exonuclease III-aided target recycling strategy for sequence-specific detection of femtomolar DNA, Biosens. Bioelectron. 28 (2011) 232–238.
[12] Y. Chen, Q. Wang, J. Xu, Y. Xiang, R. Yuan, Y.Q. Chai, A new hybrid signal amplification strategy for ultrasensitive electrochemical detection of DNA based on enzyme-assisted target recycling and DNA supersandwich assemblies, Chem. Commun. 49 (2013) 2052–2054.
[13] J. Zhang, S.P. Song, L.Y. Zhang, L.H. Wang, H.P. Wu, D. Pan, C.H. Fan, Sequence-specific detection of femtomolar DNA via a chronocoulometric DNA sensor (CDS): effects of nanoparticle-mediated amplification and nanoscale control of DNA assembly at electrodes, J. Am. Chem. Soc. 128 (2006) 8575–8580.
[14] M. Labib, N. Khan, S.M. Ghobadloo, J. Cheng, J.P. Pezacki, M.V. Berezovski, Three-mode electrochemical sensing of ultralow microRNA levels, J. Am. Chem. Soc. 135 (2013) 3027–3038.
[15] E. Farjami, R. Campos, J.S. Nielsen, K.V. Gothelf, J. Kjems, E.E. Ferapontova, RNA Aptamer-based electrochemical biosensor for selective and label-free analysis of dopamine, Anal. Chem. 85 (2013) 121–128.
[16] H.F. Dong, J.P. Lei, L. Ding, Y.Q. Wen, H.X. Ju, X.J. Zhang, MicroRNA: function, detection, and bioanalysis, Chem. Rev. 113 (2013) 6207–6233.
[17] B.V. Chikkaveeraiah, A.A. Bhirde, N.Y. Morgan, H.S. Eden, X.Y. Chen, Electrochemical immunosensors for detection of cancer protein biomarkers, ACS Nano 6 (2012) 6546–6561.
[18] J. Zheng, A.L. Jiao, R.H. Yang, H.M. Li, J.S. Li, M.L. Shi, C. Ma, Y. Jiang, L. Deng, W.H. Tan, Fabricating a reversible and regenerable raman-active substrate with a biomolecule-controlled DNA nanomachine, J. Am. Chem. Soc. 134 (2012) 19957–19960.
[19] K.J. Cash, F. Ricci, K.W. Plaxco, An electrochemical sensor for the detection of protein-small molecule interactions directly in serum and other complex matrices, J. Am. Chem. Soc. 131 (2009) 6955–6958.
[20] G.M. Nie, Z.M. Bai, W.Y. Yu, Electrochemiluminescence biosensor based on conducting poly(5-formylindole) for sensitive detection of ramos cells, Biomacromolecules 14 (2013) 834–840.
[21] R.C. Bailey, G.A. Kwong, C.G. Radu, O.N. Witte, J.R. Heath, DNA-encoded antibody libraries: a unified platform for multiplexed cell sorting and detection of genes and proteins, J. Am. Chem. Soc. 129 (2007) 1959–1967.
[22] S.J. Liu, H.G. Nie, J.H. Jiang, G.L. Shen, R.Q. Yu, Electrochemical sensor for mercury(II) based on conformational switch mediated by interstrand cooperative coordination, Anal. Chem. 81 (2009) 5724–5730.
[23] A. Abi, E.E. Ferapontova, Unmediated by DNA electron transfer in redox-labeled DNA duplexes end-tethered to gold electrodes, J. Am. Chem. Soc. 134 (2012) 14499–14507.
[24] A. Vallee-Belisle, F. Ricci, T. Uzawa, F. Xia, K.W. Plaxco, Bioelectrochemical switches for the quantitative detection of antibodies directly in whole blood, J. Am. Chem. Soc. 134 (2012) 15197–15200.
[25] M.V. Golynskiy, W.F. Rurup, M. Merkx, Antibody detection by using a FRET-based protein conformational switch, ChemBioChem 11 (2010) 2264–2267.
[26] T. Ihara, T. Ishii, N. Araki, A.W. Wilson, A. Jyo, Silver ion unusually stabilizes the structure of a parallel-motif DNA triplex, J. Am. Chem. Soc. 131 (2009) 3826–3827.

Please cite this article in press as: W. Wei, et al., Fabricating a reversible and regenerable electrochemical biosensor for quantitative detection of antibody by using "triplex-stem" DNA molecular switch, Anal. Chim. Acta (2014), http://dx.doi.org/10.1016/j.aca.2014.07.015

[27] J. Zheng, J.S. Li, Y. Jiang, J.Y. Jin, K.M. Wang, R.H. Yang, W.H. Tan, Design of aptamer-based sensing platform using triple-helix molecular switch, Anal. Chem. 83 (2011) 6586–6592.
[28] D.P. Arya, R.L. Coffee, I. Charles, Neomycin-induced hybrid triplex formation, J. Am. Chem. Soc. 123 (2001) 11093–11094.
[29] F.M. Chen, Intramolecular triplex formation of the purinee.purine.pyrimidine type, Biochemistry 30 (1991) 4472–4479.
[30] C.H. Wang, C. Yang, Y.Y. Song, W. Gao, X.H. Xia, Adsorption and direct electron transfer from hemoglobin into a three-dimensionally ordered. Macroporous gold film, Adv. Func. Mater. 15 (2005) 1267–1275.

Please cite this article in press as: W. Wei, et al., Fabricating a reversible and regenerable electrochemical biosensor for quantitative detection of antibody by using “triplex-stem” DNA molecular switch, Anal. Chim. Acta (2014), http://dx.doi.org/10.1016/j.aca.2014.07.015