



Contents lists available at ScienceDirect

Biomaterials

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

A hairpin aptamer-based electrochemical biosensing platform for the sensitive detection of proteins

Zai-Sheng Wu, Fan Zheng, Guo-Li Shen*, Ru-Qin Yu*

State Key Laboratory for Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, PR China

ARTICLE INFO

Article history:

Received 29 November 2008

Accepted 2 February 2009

Available online xxx

Keywords:

Aptamer probe

Conformational change

Electron-transfer distance

Alternating current voltammetry

ABSTRACT

An aptamer-based electrochemical sensing platform for the direct protein detection has been developed using IgE and a specifically designed aptamer with hairpin structure as the model analyte and probe sequence, respectively. In the absence of IgE, the aptamer immobilized on an electrode surface forms a large hairpin due to the hybridization of the two complementary arm sequences, and peak currents of redox species dissolved in solution can be achieved. However, the target protein binding can not only cause the increase of the dielectric layer but also trigger the significant conformational switching of the aptamer due to the opening of the designed hairpin structure that pushes the biomolecule layer/electrolyte interface away from the electrode surface, suppressing substantially the electron transfer (eT) and resulting in a strong detection signal. The detection limit of 3.6×10^{-11} M and linear response range of 5.4×10^{-11} to 3.6×10^{-8} M are achieved without any amplifier. The selectivity is confirmed by interference test. More importantly, an innovative concept of adapting intelligently a surface-confined aptamer sequence is introduced, and the limitations of the conventional electrochemical aptasensors have been overcome. The proposed sensing scheme is expected to become a promising strategy for the detection of proteins and other biomacromolecules.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Aptamers are short, single-stranded, functional DNA or RNA molecules selected from random-sequence nucleic acid combinatorial libraries by Systematic Evolution of Ligands by Exponential Enrichment (SELEX). The aptamers are chemically stable, small in size, inexpensive and can bind nearly any target with high affinity and specificity [1]. The flexibility and convenience in the design of aptamer sequences make them attractive bio-recognition elements. Despite the efforts of a few groups to develop simple and convenient colorimetric aptamer-based assay methods, sensitive target detections using colorimetry proved rather difficult [2,3]. Although an impressive number of fluorescence-detected signaling aptamers have been reported [4–9], the applications of those fluorescent signaling aptamers remain challenging to a certain extent [6,8,9]. Electrochemical detection offers possibly an alternative to optical techniques for the development of aptamer-based assays. An increasing effort has been recently devoted to the design of electrochemical aptamer-based detection systems, and impressive results have been achieved [10–17].

Immunoglobulin E (IgE), only been found in mammals, is a class of antibody (or immunoglobulin “isotype”) in biology. Although the IgE concentration is extremely low in human serum (about 1 nM) in a normal (“non-atopic”) individual, it can trigger the most powerful immune reactions [18]. However, serum IgE is significantly raised in patients afflicted with allergic asthma, atopic dermatitis and other immune deficiency-related diseases, including AIDS [19]. Therefore, the IgE determination is of great value. Electrochemical aptamer-based biosensor using a redox-tagged, surface-confined aptamer as the probe sequence for the label-free reagentless detection of specific target molecule has proven versatile and received an increasing amount of attention [10–15]. However, there is no demonstration of an analytical paradigm for the IgE detection thought some aptamer-based assays have been reported utilizing distinct signal transduction mechanisms [19–23]. As indicated in a literature [16], to construct a reagentless sensing interface, a large-scale conformational change upon the target binding is required as the binding signal arises from the motion of terminal redox-active species relative to the external substrate surface. Thus, the probable reason for the lack of reagentless electrochemical biosensor for the label-free IgE detection is that the conformational change of the standard aptamer sequence upon target binding is not large enough to generate an electrochemical signal. Possibly by the same token, other types of electrochemical assay systems for

* Corresponding authors. Tel./fax: +86 731 8821355.

E-mail addresses: glshen@hnu.cn (G.-L. Shen), rquyu@hnu.net.cn (R.-Q. Yu).

the IgE detection are very few. To fabricate a sensitive sensing interface, the key is to design a versatile aptamer probe that can undergo significant conformation change to transduce efficiently target protein binding events to a detectable signal.

In the present contribution, a new electrochemical aptasensor is described based on the conformational change of a specifically designed DNA aptamer sequence, in which human immunoglobulin E (IgE) is used as the target protein. The thiolated 81-mer aptamer containing the original sequence of 37-mer anti-IgE aptamer consensus probe is assembled on the surface of a gold electrode via sulfur–gold affinity and can fold into a loop–stem structure as the two complementary arm sequences hybridize with each other. When being immersed in a redox-active solution, the aptamer-modified gold electrode can offer a significant peak current. In the presence of target protein, the interaction between the recognition motif of the designed aptamer and IgE induces a dramatic decrease in peak current without any amplifier. Using the present electrochemical aptamer-based assay platform, the IgE detection can be readily accomplished, and a relatively low detection limit as well as a wide linear dynamic range can be obtained. The detailed scheme of the electrochemical protein detection is represented; the conditions for the preparation of the sensing interface are investigated, and the analytical characteristics achieved are evaluated.

2. Materials and methods

2.1. Reagents

The anti-IgE aptamer used in the present study was designed and evaluated with the help of DNA folding software based on the previous works [24,25] and synthesized by Takara Biotechnology Co. (Dalian, China). The base sequence for the aptamer is as follows:

5′-HS-(CH₂)₆-GCGCG GGGCA CGTCA TAACC TTCAG CAAGC TTAA CTCAG GGGCA CGTT ATCCG TCCCT CCTAG TGGCG TGCCG CGCCG-3′.

The underlined fragments refer to the two complementary arm sequences; the italicized fragment is the random spacer sequence; the bold fragment is the standard anti-IgE aptamer sequence. Immunoglobulin E (IgE) purified from human plasma was purchased from Meridian Life Science, Inc. (Saco, ME, USA). Bovine serum albumin (BSA), human serum albumin (HSA) and human IgG were obtained from Beijing Dingguo Biotechnology Center (Beijing, China). The aptamer and protein solutions were prepared with 10 mM phosphate buffered saline (PBS, pH 7.4) containing 2.7 mM KCl, 200 mM NaCl and 1 mM MgCl₂ and stored at 4 °C for future use.

All other chemicals were of analytical grade and used as-received without further purification. Deionized and autoclaved water (resistance > 18 MΩ/cm) was used throughout the experiments.

2.2. Aptamer-based biosensor preparation

A gold electrode (1.0 mm diameter) received from Chenhua Instruments Co. (Shanghai, China) was polished to a mirror smoothness successively with 1.0, 0.3 and 0.05-μm alumina powder on a clean polishing microcloth. After each polish, the electrode was thoroughly washed with water. After sonicating in water, absolute alcohol and water for 5 min each, the resulting electrode was incubated with piranha solution (30% H₂SO₄/70% H₂O₂, WARNING: piranha solution reacts violently with organic materials) for 20 min. Following washing with water, the electrode was electrochemically treated by potential cycling in 0.1 M H₂SO₄ until stable voltammograms could be obtained. After being washed with water again, the cleaned gold electrode was dried in a stream of nitrogen.

To fabricate an aptamer-based biosensor, 10 μL of 1.2 μM aptamer solution was given onto the surface of the inverted cleaned electrode, and the self-assembly reaction was allowed to proceed in a humidity chamber at room temperature for 120 min. Subsequently, the aptamer-modified electrode was washed with copious water to remove the non-immobilized aptamer probes. After being exposed to a 1 M glycine solution for 30 min to block the remaining bare region, the resulting electrode was ready for the IgE detection.

2.3. Electrochemical measurements

Alternating current (AC) impedance, AC voltammetric and cyclic voltammetric (CV) measurements were carried out in a conventional three-electrode setup using CHI 760B electrochemical workstation (Shanghai, China). The counter electrode was a large area platinum foil while the reference electrode was a saturated calomel

electrode (SCE, saturated KCl), against which all potentials were reported. Impedance spectra were recorded over a voltage frequency range of 1–100,000 Hz at an applied potential of 240 mV with the AC voltage amplitude of 5 mV. CV experiments were conducted in the scan range from −0.2 to +0.6 V at a scan rate of 100 mV/s. Aptamer-based biosensors were evaluated using AC voltammetry over the potential range from −0.2 to 0.7 V at a frequency of 1 Hz, a sample period of 1.1 s and an amplitude of 25 mV. All electrochemical measurements were performed in 10 mM PBS (pH 7.4) containing 0.1 M KCl and 5 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆] redox couple at room temperature.

2.4. Procedure of protein detection

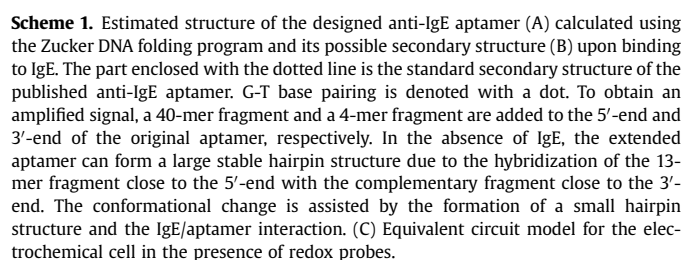
A 10-μL droplet of IgE solution at specific concentration was placed on the surface of the aptamer-modified electrode, and the target–aptamer binding was allowed to react for 60 min. After rinsing with PBS, the AC voltammetric measurement was performed. The peak current was used to evaluate the target protein content in sample as it was determined by the amount of IgE accumulated on the modified electrode surface that was directly related to the target concentration.

3. Results and discussion

3.1. Aptamer probe design

For a given reversible reaction, the voltammogram can provide information not only about the formal potential of the surface redox reaction but also about the amount of redox-active species. On the other hand, as shown in the literatures [26,27], the rate constant (k_{et}) of electron transfer (eT) across self-assembled monolayers with functional redox molecules or reactants dissolved in solution is measured as a function of the eT distance (L). In general, for nonresonant tunneling, the eT rate constant decreases exponentially with increasing L . The values of the decay constant $\beta(=-\ln[k_{et}]/dL)$ range from 0.2 to 0.6 Å^{−1} for unsaturated bridges and 0.8–1.5 Å^{−1} for saturated ones. Under specific conditions, the peak intensity is determined by the electron-transfer distance between the redox species and the electrode surface. To some extent, these works provide hints for the electrochemical assay systems based on the conformational change of surface-confined aptamer DNA sequences.

On the basis of the foregoing considerations, an electrochemical aptamer-based biosensor via target-induced conformation switching of specifically designed aptamer is presented in the present contribution. As reported in the earlier literatures, the binding properties of an aptamer depend extremely on its intact base sequence, especially in the loop [28,29]. The adaptation of the loop sequence of aptamer probes might cause a nearly complete loss of binding activity [29,30]. To make the aptamer undergo a large-scale conformational change upon target binding without sacrificing its binding capability, we designed specifically an aptamer probe against IgE with a 13-base-pair self-complementary stem and a 55-base loop via lengthening the original aptamer sequence by 44 bases. The sequence and secondary structure of the synthetic aptamer are shown in Scheme 1A and B. One can see that this extended aptamer sequence contains a standard aptamer segment that can specifically interact with target IgE as shown in the previous works [20,21,23]. The electrochemical detection of target IgE using the designed aptamer-based biosensor (as opposed to an assay system based on a standard anti-IgE aptamer sequence) is shown in Scheme 2. As the perfectly matched stem 1 is longer than the stem 2 containing an inserted base, the designed aptamer probe can fold into a large hairpin structure in the absence of target protein. In this case, a high electron-transfer rate for the redox reaction of [Fe(CN)₆]^{3−/4−} is obtained. However, the IgE binding can induce the structure switching of the aptamer probe from a large hairpin to a protein/aptamer complex, resulting in an increase not only in the dielectric constant of the bio-recognition layer but also in the electron-transfer distance as the partial stretching of the aptamer probe pushes the biomolecule/electrolyte interface away



Scheme 2. Schematic representation of the construction of electrochemical aptamer-based biosensor and the principle of IgE detection. The designed aptamer probe, a 5'-thiolate DNA sequence, can fold into a hairpin structure and is immobilized onto a gold electrode surface. Short vertical lines denote glycine molecules used to block the bare region of gold electrode. The bases in the loop region are not shown. The target binding not only increases the dielectric constant of the bio-recognition layer but also induces the conformational change of the designed aptamer, suppressing significantly the electron transfer (eT) that triggers a current response.

capability. The observation was consistent with the fact that electroactive moieties could not efficiently exchange electrons with the separation distance of more than 10 nm [12].

3.2. Time dependence of the self-assembly of the designed aptamer

The incubation time for the self-assembly of the designed aptamer was explored as it directly influence the immobilization efficiency. Fig. 1B describes the time dependence of the peak current decay recorded for the modified interface prepared by exposure of a freshly cleaned gold electrode to the aptamer solution for different periods of time. The peak current decreases rapidly with the increment of assembly time up to 60 min, and then gradually reach a plateau, indicating that the self-assembly reaches a steady state. Although a coverage efficiency of about 95% is obtained at 60 min assuming that the coverage efficiency of the used aptamer is 100% for the maximal current change, 120-min incubation time is used for the aptamer self-assembly in subsequent experiments.

3.3. Electrochemical characterization of aptamer-based biosensor

The ability of the films to insulate the electrode surface at different stages of the aptasensor preparation was investigated by cyclic voltammetry in the presence of a fairly reversible redox couple $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in solution. As shown in Fig. 2A, the cyclic voltammogram at the bare gold electrode exhibits a pair of reversible redox peaks with very high peak currents (line a);

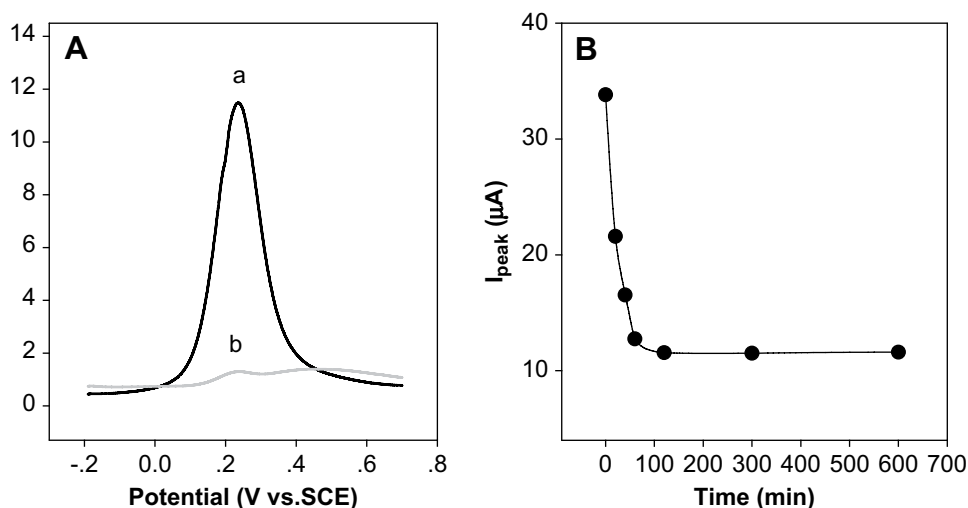


Fig. 1. (A) The current response for the aptamer-based biosensor prepared via the proposed method. Lines a and b indicate the AC voltammograms collected for the aptamer-modified electrode before and after being incubated in 36 nM IgE solution, respectively. (B) Influence of the time for the self-assembly of anti-IgE aptamer on the peak current of the proposed biosensor. The peak current of AC voltammogram recorded for the bare gold electrode was used to evaluate the initial state of the gold electrode exposed to aptamer solution.

Self-assembled monolayer of the designed aptamer probe on the electrode surface leads to a large decrease of the peak currents as well as an increase in the peak-to-peak separation (line b). This phenomenon is explained by a fact that the electrostatic repulsion between anionic $[Fe(CN)_6]^{3-/4-}$ and the surface-bound aptamer DNA sequences with negative charges retards the interfacial electron-transfer kinetics. The aptamer-modified gold electrode exposed to glycine solution does not exhibit an obvious change in the insulating property (line c). Presumably, the physically adsorbed glycine layer cannot impede the interfacial electron transfer. A large detection signal is achieved after binding of IgE to the surface-confined aptamer probe (line d), indicating that the electron transfer between the electrode surface and redox couples in solution was substantially hindered due to the opening of the stem 1 and the formation of IgE/aptamer complex (shown in Scheme 2). Additionally, we infer that the dissociation of target protein from the aptamer-modified electrode during the process of performing the cyclic voltammetric measurements is negligible since the peak

currents do not display obvious changes (not shown here) even after tens of cycles.

As an effective method to probe the interface properties of modified electrodes, impedance spectroscopy was also used to evaluate the interfacial electron-transfer efficiency at different stages of biosensor preparation. Fig. 2B displays Faradaic impedance spectra (presented as Nyquist plot) of the same electrode with different modified surfaces. Equivalent circuit model for the electrochemical cell in the presence of a redox probe, $[Fe(CN)_6]^{3-/4-}$, is shown in Scheme 1C. R_s , Z_w , R_{et} and Cdl are the ohmic resistance of the electrolyte solution, the Warburg impedance resulting from the diffusion of ions from the bulk of the electrolyte to the interface, the electron-transfer resistance and the double layer capacitance, respectively. R_s and Z_w are not affected by the chemical transformations occurring at the electrode surface while Cdl and R_{et} depend on the dielectric and insulating features at the electrode/electrolyte interface. As seen in Fig. 2B, the impedance of the aptasensor challenged with target molecules is dominated by slow long-range charge transfer across the surface-immobilized biomolecule layer [32];

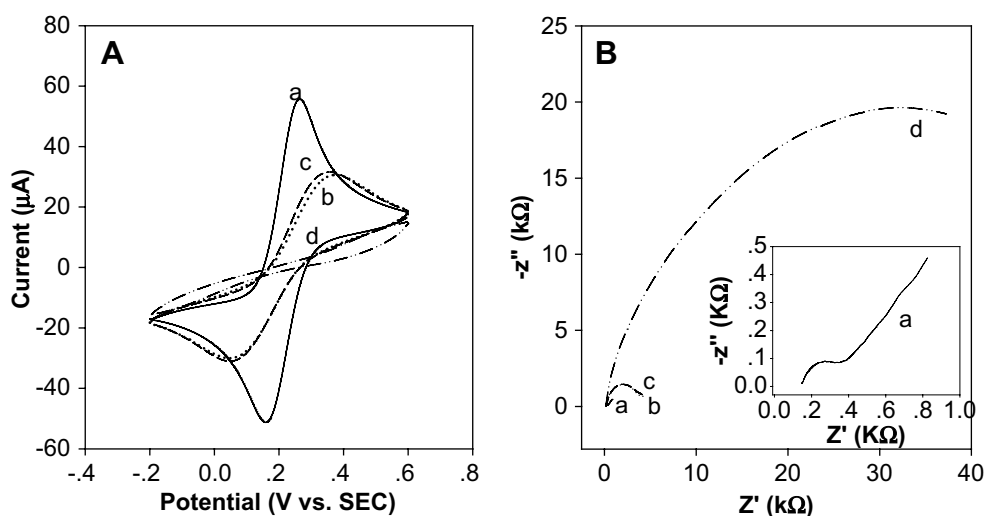


Fig. 2. Cyclic voltammograms (A) and AC impedances (B) of the aptamer-based biosensor at different preparation stages. (a) Bare gold electrode; (b) aptamer-modified gold electrode; (c) glycine-blocked gold electrode; (d) aptamer-based sensing interface after interaction with IgE at 36 nM for 1 h.

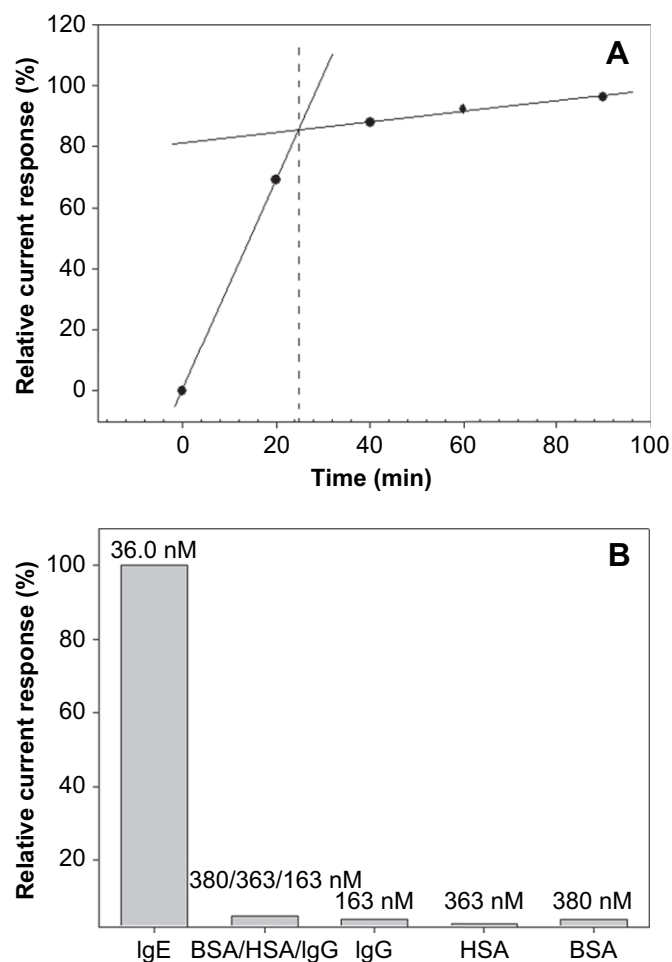


Fig. 3. (A) Effect of incubation time for the aptamer/IgE interaction on the current signal of the aptamer-based biosensor. The peak current of AC voltammogram recorded before the addition of IgE was used to estimate the initial state of the sensing interface obtained by being incubated in the analyte-containing sample. IgE solution at the concentration of 36 nM was involved. (B) Selectivity of the proposed assay platform. The IgG (163 nM), HSA (363 nM), BSA (380 nM) and a mixture of 163 nM IgG, 363 nM HSA and 380 nM BSA were used to evaluate the detection selectivity. A blank was obtained after incubating the working electrode in the absence of IgE under identical conditions. The electrochemical signals presented are the blank-subtracted data.

An 13-fold increase in the imaginary part of the Faradaic impedance and 8-fold increase in the real part were observed upon the target protein–aptamer interaction. Even though the impedance biosensors are based on aptamer probes against other proteins that display an obvious binding-induced conformational change [11,13], the increase in the electron-transfer resistances upon the interaction between the aptamer and target is lower than 4-fold [33,34].

The measured data indicated that the target protein binding could achieve a substantial change in the electrochemical behavior of surface-confined biomolecule layer. This phenomenon should be attributed to both the significant conformational change of aptamer probes and the formation of target–aptamer complexes. Obviously, the newly designed aptamer-based sensor could be used for the sensitive detection of IgE, and the analytical performance of other aptamer-based electrochemical sensors is also expected to be improved if the aptamer sequence is adapted by the present strategy.

3.4. Incubation time for IgE–aptamer binding

The incubation time for the interaction of target protein and the surface-bound aptamer probe was investigated as a factor influencing

the analytical performance of the present biosensor. Fig. 3A shows a plot of the relative current response as a function of the incubation time of target protein. One can see that the relative current response increases rapidly upon target binding and then changes slowly after about 25 min (the point formed by two straight lines). In our work, 60-min incubation time was chosen for binding of target protein to the aptamer-modified electrode in subsequent experiments.

3.5. Selectivity study

To confirm that the current signal is induced by the specific interaction of the novel designed aptamer and the target molecule, the degree of the nonspecific binding of other proteins was evaluated under the same experimental conditions as in the case of IgE. The experimental results are shown in Fig. 3B. The mixture of 163 nM IgG, 363 nM HSA and 380 nM BSA can induce a relative current response of 5% based on the assumption that the specific binding response upon addition of target IgE is 100%. The influence of these proteins was also independently investigated. The current change induced by HSA and BSA is small. Although the structure of IgG is similar to that of target IgE [20], the relative current response caused by this molecule is about 3% even at a higher concentration than that of IgE. These measured data indicates an insignificant cross-reactivity. It is clear that the extended aptamer probe with specifically designed hairpin structure by adapting intelligently the published anti-IgE aptamer can bind its target molecule with high affinity and selectivity. It is reasonable that the recognition motifs of the designed aptamers can maintain their binding properties since the chemical modification of aptamers with external labeling groups does not disrupt the formation of aptamer–target complexes when the labels are far away from the recognition motifs [35]. These data obtained provided direct evidence that the current signal is generated from the specific interaction between the aptamer and IgE.

3.6. Analytical characteristics of aptamer-based biosensor

To ensure the present assay platform can be used for sensitive quantification of target protein, the current responses induced by IgE at different concentrations are evaluated. As the IgE concentration increases, the resultant electron-transfer resistance is gradually enhanced, yielding an electrochemical signal. As shown

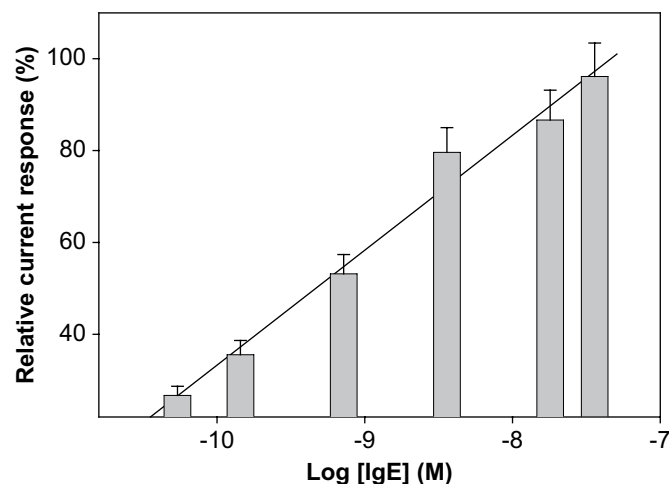


Fig. 4. The linear relationship between the relative current decrease and Log IgE concentration. The error bars indicated the standard deviation of triplicate determinations for each concentration of IgE. The regression equation was $Y = 25.0 \log X + 283.6$ with a correlation coefficient of 0.9858, where Y and X represented the relative current response and the IgE concentration, respectively.

in Fig. 4, there is a linear relationship between the relative current response and the logarithm of the target concentration in the range of 5.4×10^{-11} to 3.6×10^{-8} M. There is no significant change in recognition signal when the target concentration increases or decreases further. The points at higher or lower target concentration are not within the linear response range. To evaluate the applicability and reliability of the proposed system, the recovery experiment was carried out. Four samples containing the same concentration (3.6×10^{-9} M) were prepared individually, and each was detected four times. The average recovery was $\sim 97\%$ with the average relative standard deviation of 8.9%. The detection limit was 3.6×10^{-11} M, at which target protein binding can trigger a well-defined current signal when compared with the blank current. Taking the sample volume (10 μ L) into consideration, the present aptasensor is capable of efficiently detecting down to 360 amol of IgE, indicating that the detection capability achieved is much better than that of fluorescence assay using signaling aptamer as the probe [20,21]. Using the proposed strategy, a wider linear response range and a lower detection limit can be achieved compared to the existing electrochemical biosensors using a standard aptamer DNA sequence as the probe molecule [19,30].

4. Conclusions

A new strategy to convert efficiently the aptamer–target recognition event into an electrochemical signal was described in the present work by introducing an additional stem and a spacer fragment into the standard aptamer sequence. The extended aptamer can maintain the binding properties of the original sequence and preserve high affinity and selectivity, ensuring that the corresponding detection system possesses attractive features, for instance, wide linear response range, low detection limit and high selectivity. The present electrochemical direct sensing platform exhibits several advantages: a simple design of an aptamer sequence, low-cost and straightforward fabrication, and almost effortless detection procedure. It is worth noting that an innovative probe concept is introduced in this contribution. Using the present strategy, various assay platforms could be readily constructed based on the target binding-induced large-scale conformational changes of aptamer probes even though the conformation of the standard aptamer sequence change slightly upon target binding, thus holding great promise and potential for applications in diagnostics and proteomics.

Acknowledgements

The work was financially supported by National Basic Research Program (Grant No. 2007CB310500) and the National Natural Science Foundation of China (Grant No. 20675028 and 20775023).

Appendix

Figures with essential colour discrimination. Scheme 2 in this article may be difficult to interpret in black and white. The full colour version can be found in the on-line version, at [doi:10.1016/j.biomaterials.2009.02.017](https://doi.org/10.1016/j.biomaterials.2009.02.017).

References

- [1] Song S, Wang L, Li J, Fan C, Zhao J. Aptamer-based biosensors. *Trends Anal Chem* 2008;2:108–17.
- [2] Liu J, Lu Y. Fast colorimetric sensing of adenosine and cocaine based on a general sensor design involving aptamers and nanoparticles seleno-modified RNA. *Angew Chem* 2006;118:96–100.
- [3] Liu J, Lu Y. Adenosine-dependent assembly of aptazyme-functionalized gold nanoparticles and its application as a colorimetric biosensor. *Anal Chem* 2004;76:1627–32.
- [4] Yamana K, Ohtani Y, Nakano H, Saito I. Bis-pyrene labeled DNA aptamer as an intelligent fluorescent biosensor. *Bioorg Med Chem Lett* 2003;13:3429–31.
- [5] Merino EJ, Weeks KM. Facile conversion of aptamers into sensors using a 2'-ribose-linked fluorophore. *J Am Chem Soc* 2005;127:12766–7.
- [6] Nutiu R, Li Y. Structure-switching signaling aptamers. *J Am Chem Soc* 2003;125:4771–8.
- [7] Stojanovic MN, de Prada P, Landry DW. Aptamer-based folding fluorescent sensor for cocaine. *J Am Chem Soc* 2001;123:4928–31.
- [8] Yang CJ, Jockusch S, Vicens M, Turro NJ, Tan W. Light-switching excimer probes for rapid protein monitoring in complex biological fluids. *Proc Natl Acad Sci U S A* 2005;102:17278–83.
- [9] Stojanovic MN, de Prada P, Landry DW. Fluorescent sensors based on aptamer self-assembly. *J Am Chem Soc* 2000;122:11547–8.
- [10] Baker BR, Lai RY, Wood MS, Doctor EH, Heeger AJ, Plaxco KW. An electronic, aptamer-based small-molecule sensor for the rapid, label-free detection of cocaine in adulterated samples and biological fluids. *J Am Chem Soc* 2006;128:3138–9.
- [11] Radi AE, Sanchez JLA, Baldrich E, O'Sullivan CK. Reagentless, reusable, ultra-sensitive electrochemical molecular beacon aptasensor. *J Am Chem Soc* 2006;128:117–24.
- [12] Zuo X, Song S, Zhang J, Pan D, Wang L, Fan C. A Target-Responsive Electrochemical Aptamer Switch (TREAS) for reagentless detection of nanomolar ATP. *J Am Chem Soc* 2007;129:1042–3.
- [13] Lai RY, Plaxco KW, Heeger AJ. Aptamer-based electrochemical detection of picomolar platelet-derived growth factor directly in blood serum. *Anal Chem* 2007;79:229–33.
- [14] Xiao Y, Piorek BD, Plaxco KW, Heeger AJ. A reagentless signal-on architecture for electronic, aptamer-based sensors via target-induced strand displacement. *J Am Chem Soc* 2005;127:17990–1.
- [15] Radi AE, O'Sullivan CK. Aptamer conformational switch as sensitive electrochemical biosensor for potassium ion recognition. *Chem Commun* 2006:3432–4.
- [16] Lu Y, Li X, Zhang L, Yu P, Su L, Mao L. Aptamer-based electrochemical sensors with aptamer-complementary DNA oligonucleotides as probe. *Anal Chem* 2008;80:1883–90.
- [17] Wu Z-S, Guo M-M, Zhang S-B, Chen C-R, Jiang J-H, Shen G-L, et al. Reusable electrochemical sensing platform for highly sensitive detection of small molecules based on structure-switching signaling aptamers. *Anal Chem* 2007;79:2933–9.
- [18] Sutton BJ, Gould HJ. The human IgE network. *Nature* 1993;366:421–8.
- [19] Maehashi K, Katsura T, Kerman K, Takamura Y, Matsumoto K, Tamiya E. Label-free protein biosensor based on aptamer-modified carbon nanotube field-effect transistors. *Anal Chem* 2007;79:782–7.
- [20] Gokulrangan G, Unruh JR, Holub DF, Ingram B, Johnson CK, Wilson GS. DNA aptamer-based bioanalysis of IgE by fluorescence anisotropy. *Anal Chem* 2005;77:1963–70.
- [21] Jiang Y, Fang X, Bai C. Signaling aptamer/protein binding by a molecular light switch complex. *Anal Chem* 2004;76:5230–5.
- [22] German I, Buchanan DD, Kennedy RT. Aptamers as ligands in affinity probe capillary electrophoresis. *Anal Chem* 1998;70:4540–5.
- [23] Liss M, Petersen B, Wolf H, Prohaska E. An aptamer-based quartz crystal protein biosensor. *Anal Chem* 2002;74:4488–95.
- [24] Zuker M. M fold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res* 2003;31:3406–15.
- [25] SantaLucia J. A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics. *Proc Natl Acad Sci U S A* 1998;95:1460–5.
- [26] Wu Z-S, Chen C-R, Shen G-L, Yu R-Q. Reversible electronic nanoswitch based on DNA G-quadruplex conformation: a platform for single-step, reagentless potassium detection. *Biomaterials* 2008;29:2689–96.
- [27] Liu B, Bard AJ, Mirkin MV, Creager SE. Electron transfer at self-assembled monolayers measured by scanning electrochemical microscopy. *J Am Chem Soc* 2004;126:1485–92.
- [28] Wiegand TW, Williams PB, Dreskin SC, Jouvin MH, Kinet JP, Tasset D. High-affinity oligonucleotide ligands to human IgE inhibit binding to Fc epsilon receptor 1J. *Immunology* 1996;157:221–30.
- [29] Katilius E, Flores C, Woodbury NW. Exploring the sequence space of a DNA aptamer using microarrays. *Nucleic Acids Res* 2007;35:7626–35.
- [30] Xu D, Xu D, Yu X, Liu Z, He W, Ma Z. Label-free electrochemical detection for aptamer-based array electrodes. *Anal Chem* 2005;77:5107–13.
- [31] Steel AB, Herne TM, Tarlov MJ. Electrochemical quantitation of DNA immobilized on gold. *Anal Chem* 1998;70:4670–7.
- [32] Pan S, Rothberg L. Chemical control of electrode functionalization for detection of DNA hybridization by electrochemical impedance spectroscopy. *Langmuir* 2005;21:1022–7.
- [33] Liao W, Cui XT. Reagentless aptamer based impedance biosensor for monitoring a neuro-inflammatory cytokine PDGF. *Biosens Bioelectron* 2007;23:218–24.
- [34] Radi A-E, Acero Sanchez JL, Baldrich E, O'Sullivan CK. Reusable impedimetric aptasensor. *Anal Chem* 2005;77:6320–3.
- [35] Fang X, Cao Z, Beck T, Tan W. Molecular aptamer for real-time oncoprotein platelet-derived growth factor monitoring by fluorescence anisotropy. *Anal Chem* 2001;73:5752–7.