Biosensors and Bioelectronics 39 (2013) 133–138

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Sensitive label-free electrochemical analysis of human IgE using an aptasensor with cDNA amplification

Cheng-Yu Lee, Kuan-Ying Wu, Hsiu-Li Su, Huan-Yi Hung, You-Zung Hsieh*

Department of Applied Chemistry, National Chiao Tung University, Hsinchu, Taiwan, ROC

ARTICLE INFO

Article history:
Received 26 April 2012
Received in revised form
6 July 2012
Accepted 7 July 2012
Available online 24 July 2012

Keywords:
Label-free electrochemical aptasensor
cDNA amplification
Methylene blue
Human IgE

ABSTRACT

In this study, we developed an ultrasensitive label-free aptamer-based electrochemical biosensor, featuring a highly specific anti-human immunoglobulin E (IgE) aptamer as a capture probe, for human IgE detection. Construction of the aptasensor began with the electrodeposition of gold nanoparticles (AuNPs) onto a graphite-based screen-printed electrode (SPE). After immobilizing the thiol-capped anti-human IgE aptamer onto the AuNPs through self-assembly, we treated the electrode with mercaptohexanol (MCH) to ensure that the remaining unoccupied surfaces of the AuNPs would not undergo nonspecific binding. We employed a designed complementary DNA featuring a guanine-rich section in its sequence (cDNA G1) as a detection probe to bind with the unbound anti-human IgE aptamer. We measured the redox current of methylene blue (MB) to determine the concentration of human IgE in the sample. When the aptamer captured human IgE, the binding of cDNA G1 to the aptamer was inhibited. Using cDNA G1 in the assay greatly amplified the redox signal of MB bound to the detection probe. Accordingly, this approach allowed the linear range (coefficient of determination: 0.996) for the analysis of human IgE to extend from 1 to 100,000 pM; the limit of detection was 0.16 pM. The fabricated aptasensor exhibited good selectivity toward human IgE even when human IgG, thrombin, and human serum albumin were present at 100-fold concentrations. This method should be readily applicable to the detection of other analytes, merely by replacing the anti-human IgE aptamer/cDNA G1 pair with a suitable anti-target molecule aptamer and cDNA.

1. Introduction

Aptamers are synthetic DNA or RNA sequences that are selected through SELEX (systematic evolution of ligands by exponential enrichment) processes from a random sequence bank (Deng et al., 2009; Sassolas et al., 2009). High affinity and specific binding usually occur between a selected aptamer and its related target molecule. The combination of good binding affinity and excellent selectivity makes aptamers as attractive as antibodies when used as critical identification materials during protein analysis. Aptamers are, however, more stable, easier to store, and cheaper than antibodies. Furthermore, aptamers can be synthesized without using a host. Therefore, aptamers have been used as alternatives to antibodies in various fields for various applications (Hamaguchi et al., 2001; Iqbal et al., 2000; Liu and Lu, 2004; Tombelli et al., 2005).

Taking advantage of their specific affinity, several aptamer-based sensors (so-called "aptasensors") have been developed to detect small molecules (Bing et al., 2010; Du et al., 2008; Zuo et al., 2009) and proteins (Bing et al., 2010; Wu et al., 2009). In the majority of these aptamer-based analyses, the aptamers have been used merely as substitutes for antibodies, with several drawbacks remaining: the approach is time consuming (multiple procedures; long incubation); the assay requires tagging of labels for most spectroscopic detection; and it is difficult to avoid background interference and unsatisfactory derivative issues. Nevertheless, the sensitivity of electrochemical detection when using the aptameric method can be competitive with spectroscopic detection schemes. Most electrochemical schemes for the detection of target analytes on fabricated electrodes are label-free, with any potential interference during analysis minimized by modifying the surface of the electrode. Other advantages of combining electrochemical detection with aptasensors include high sensitivity, simple operation, rapid response, and portability (Hianik and Wang, 2009).

Methylene blue (MB) is a popular redox agent used in the electrochemical analysis of DNA (Wang et al., 2010); its affinity toward single-strand DNA (ssDNA) is greater than that toward double-strand DNA (dsDNA) (Farjami et al., 2010; Loaiza et al., 2005). If an analyte hybridizes with ssDNA (aptamer), it will decrease the degree of binding of complementary DNA (cDNA) to the ssDNA (aptamer), in turn decreasing the redox signal of MB

* Corresponding author. Tel.: +886 3 5731785; fax: +886 3 5723764.
E-mail address: yzhsieh@mail.nctu.edu.tw (Y.-Z. Hsieh).

0956-5663/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2012.07.009

bound to the DNA. This change in electrochemical signal from MB can be used as an indicator of hybridization. In general, the interaction of MB with ssDNA occurs specifically through the guanine bases of ssDNA (Yang et al., 2002). Thus, the MB signal will be greatly enhanced if the number of guanine bases in ssDNA is increased intentionally.

Human immunoglobulin E (IgE) is an important protein found in blood, where its concentration can be used as a marker for several allergic diseases (Gould et al., 2003; Winter et al., 2000) through regular blood tests. Unlike other immunoglobulins found in blood, the concentration of human IgE is extremely low (ca.150 ng mL^{-1}) in healthy people (Gould et al., 2003). An increase in the human IgE concentration in blood can hint at illness or approaching illness. Because low concentrations of human IgE (reaching over 10 times the normal level) can be found in blood during the early stages of certain diseases (Gould et al., 2003), highly sensitive methods for its analysis are required.

Several methods have been developed for assessing human IgE concentrations (Jiang et al., 2004; Liss et al., 2002; Maehashi et al., 2007; Wang et al., 2008; Wang et al., 2010; Xu et al., 2005), including several aptamer-based electrochemical biosensors (Chen et al., 2009; Maehashi et al., 2009; Tran et al., 2011). An electrochemical biosensor for the detection of thrombin and human IgE using pyrroquinoline quinone glucose dehydrogenase (PQQGDH) as a label provided a limit of detection (LOD) of 1 nM for human IgE (Fukasawa et al., 2009). Measuring the change in electrochemical impedance allowed an aptamer-based sensor for human IgE to reach an LOD of 0.1 nM (Xu et al., 2005). Feng et al. employed the specific interactions of conjugated alkaline phosphate and a detection probe, with enzymatic silver deposition to amplify the signal response, to enhance the sensitivity of an aptasensor to an LOD of 0.02 nM (Feng et al., 2008). Wu et al. used an aptasensor platform featuring a specifically designed aptamer having a hairpin structure as the probe to directly detect human IgE; efficiently converting the aptamer–target recognition scheme into an electrochemical signal led to an LOD of 36 pM for human IgE (Wu et al., 2009). A highly sensitive aptameric assay based on chemiluminescence (CL) detection with polystyrene beads as the amplification platform has been developed for the detection of human IgE with an extra-low LOD of 4.6 pM (Peng et al., 2011).

In this study, our goal was to develop a label-free electrochemical aptasensor platform for even more sensitive analysis of human IgE as a model protein. Our constructed electrochemical biosensor featured a thiol-terminated anti-human IgE aptamer as the capture probe and its cDNA sequence as the detection probe. The aptamer sequence has been studied previously (Wang et al., 2010; Wu et al., 2009; Xu et al., 2006). We synthesized a designed cDNA featuring a guanine-rich section at its 5′ terminus (cDNA G1) and bound it with anti-human IgE aptamer. We chose MB as the electrochemical indicator because of its specific binding to guanine bases in DNA sequence (Becker and Nordén, 1997; Kara et al., 2002; Yang et al., 2002). The redox electrochemical signal of MB can be enhanced after its binding to the guanine-rich section of the detection probe. In the presence of human IgE, the amount of cDNA G1 bound to the aptamer decreases. The higher the human IgE concentration in the sample, the lower the number of cDNA G1–aptamer complexes formed, leading to fewer bound MB molecules and a lower MB redox current. Our main aim in this present study was to evaluate the proposed aptamer-based assay as an alternative and sensitive approach for analyzing proteins.

2. Material and methods

2.1. Chemicals and reagents

All chemicals were of reagent grade or higher. All stocking and working solutions were prepared using Milli-Q de-ionized (DI) water (Millipore, Bedford, MA, USA) having a measured resistance of greater than 18.2 MΩ cm. Anti-human IgE aptamer, cDNA G1, and cDNA were purchased from MDBio (Taipei, Taiwan); their sequences are listed in Table 1. Hydrogen tetrachloroaurate trihydrate were obtained from Alfa Aesar (Ward Hill, MA, USA). Sodium dihydrogen phosphate, disodium hydrogen phosphate, Tris (base), mercaptohexanol (MCH), tris(2-carboxyethyl)phosphine (TCEP), ethylenediaminetetraacetic acid (EDTA), MB, and sodium chloride were purchased from Sigma (St. Louis, MO, USA). Potassium hexacyanoferrate was obtained from Riedel–de Haën (Morristown, NJ, USA). Disposable graphite-based screen-printed electrodes (SPEs) were purchased from Zensor (Taichung, Taiwan).

2.2. Apparatus

Square-wave voltammetry (SWV), cyclic voltammetry (CV) and amperometry were performed using a CHI 8021B electrochemical analyzer (CH Instruments, Austin, TX, USA). Electrochemical impedance spectroscopy (EIS) was performed using a CHI 6081C electrochemical analyzer (CH Instruments, Austin, TX, USA). A three-electrode system was used with Ag/AgCl as the reference electrode, a platinum wire as an auxiliary electrode, and an SPE-based electrode as a working electrode. Field emission scanning electron microscopy (FE-SEM) was performed using a JSM-7401F instrument (JEOL, Tokyo, Japan).

2.3. Fabrication of aptasensor

Prior to modification of the electrode surface, the SPE was revived through 10 cycles of CV between −0.6 V to 1.2 V at 0.1 V/s in 10 mM phosphate buffer (pH 7.4). The graphite-based SPE was first modified with gold nanoparticles (AuNPs) using a procedure described previously (Liao and Ho 2009). Briefly, the revived SPE was rinsed with DI water twice and placed into 10 mM $HAuCl_4$. AuNPs were electrodeposited by applying a fixed electrode potential at −0.66 V for 10 s. After electrodeposition, the electrode was rinsed with DI water and 10 mM Tris buffer (pH 7.4). The fabricated AuNP-modified SPE was characterized using SEM and electrochemistry.

The anti-human IgE aptamer was immobilized on the surface of the electrode through self-assembly of the thiol group at the 5′ terminus of the aptamer with the AuNPs. A droplet (5 μL) of 1 μM human IgE aptamer solution, containing 0.1 mM TCEP, 1 mM EDTA, and 1 M KCl, in 10 mM Tris buffer (pH 7.4) was placed on the surface of the AuNP-modified SPE. After 12 h, the electrode

Table 1
Sequences of the aptamer and cDNAs used in this study.

Name	Sequence (5′–3′)
Anti-human IgE aptamer	HS-$(CH_2)_6$-GGG GCA CGT TTA TCC GTC CCT CCT AGT GGC GTG CCC C
cDNA G1	GGA GGA GGA GGA GGA GGA GGA GGA GGA GGA GGA GGA G GGG GCA CGC CAC TAG GAG GGA CGG ATA AAC GTG CCC C
cDNA	GGG GCA CGC CAC TAG GAG GGA CGG ATA AAC GTG CCC C

was rinsed with 10 mM Tris buffer (pH 7.4) containing 1 M KCl and then it was kept in the same solution. The aptamer-immobilized electrode was treated with 100 μM MCH for 15 min and rinsed again prior to use.

2.4. Assay of aptasensor

The detection and quantitation of human IgE was performed by adding cDNA G1, which had a sequence complementary to that of the aptamer and competed with human IgE for the immobilized anti-human IgE aptamer on the surface of the electrode. The human IgE was mixed with 10 mM Tris buffer containing 2 μM of cDNA G1 and 1 M KCl. A droplet (5 μL) of the mixed solution was placed on the electrode for 12 h to ensure the equilibrium of competition reactions. Single rinse step was performed to remove excess cDNA G1 and non-bound human IgE by using 10 mM Tris buffer containing 10 mM KCl. The assay was then placed for 10 min in 10 mM Tris buffer containing 20 μM MB and 10 mM KCl before being rinsed again and placed into 10 mM Tris buffer containing 10 mM KCl for measurement. The CV system was scanned from +0.7 to −0.2 V at a scan rate of 0.1 V/s. The SWV system was scanned from −1.0 to 0 V with amplitude of 25 mV and a step potential of 4 mV at a frequency of 15 Hz. The maximum current of the signal was used to evaluate the concentration of human IgE.

3. Results and discussion

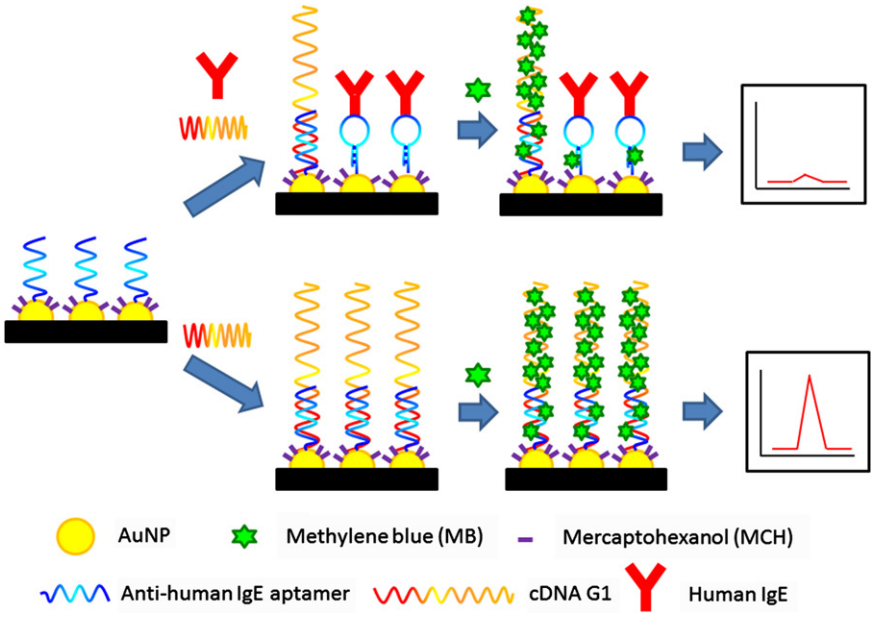
3.1. Detection principle of electrochemical aptasensor

Scheme 1 depicts the electrochemical aptasensor that we developed for the detection of human IgE. This electrochemical biosensor employed a thiol-terminated aptamer as the capture probe and a modified cDNA G1 as the detection probe. First, AuNPs were electrodeposited on a graphite-based SPE. The thiol-capped anti-human IgE aptamer was then immobilized, through self-assembly, onto the AuNPs of the SPE. To complete the construction of the aptasensor, we treated the electrode with MCH to cover any remaining unoccupied surfaces of the AuNPs, thereby preventing nonspecific binding. When the aptasensor was immersed in a sample, human IgE and cDNA G1 bound competitively with the aptamer units. We used MB as the electrochemical indicator because it bound strongly to the guanine-rich section in cDNA G1 and provided an extra redox electrochemical signal in addition to that of the cDNA. Thus, the guanine-rich component of cDNA G1 enhanced both the MB binding capacity and the redox signal.

In the absence of human IgE (bottom half of Scheme 1), the electrochemical signal from MB bound to cDNA G1 would reach its maximum value. In the presence of human IgE (top half of Scheme 1), the electrochemical signal would decrease upon decreasing the amount of cDNA G1 bound to the anti-human IgE aptamer. Thus, the difference between the redox current of MB in the presence and absence of human IgE could be used to reflect the concentration of human IgE. A higher human IgE concentration in the sample would result in a lower MB redox current. This approach is, therefore, an alternative to the aptamer-based sandwich protein assay.

3.2. Fabrication and electrochemical characterization of aptasensor

Electrochemical methods like CV and EIS were widely used to confirm the electrode fabrication process in studies involving aptamer sensor (Du et al., 2008; Wang et al., 2010; Wu et al., 2009). The responses of CV and EIS indicated not only the change in surface area but also the change in resistance and carried charge, thus providing additional information about electrode surface modification. Thus, we used CV and EIS to confirm the fabrication of the sensing interface of the aptasensor. Fig. 1(A) presents cyclic voltammograms of the aptasensor at different stages during its preparation, scanning from +0.7 to −0.2 V at a rate of 0.1 V/s for 10 cycles in the presence of a reversible redox couple, 1 mM $Fe(CN)_6^{3-/4-}$ in 1 M KCl. The cyclic voltammogram of the SPE features a pair of reversible redox peaks (line a). The SPE electrode was first modified with AuNPs, resulting in increased electrocatalytic ability, with the redox current of $Fe(CN)_6^{3-/4-}$ increasing by approximately 1.5-fold over that of the bare SPE (line b). Thus, the electrodeposited AuNPs not only improved the conductivity of the bare SPE but also provided a larger effective surface area. After immobilizing the thiol-terminated aptamer on the AuNP-modified SPE, the redox current



Scheme 1. Electrochemical aptasensor for detecting human IgE.

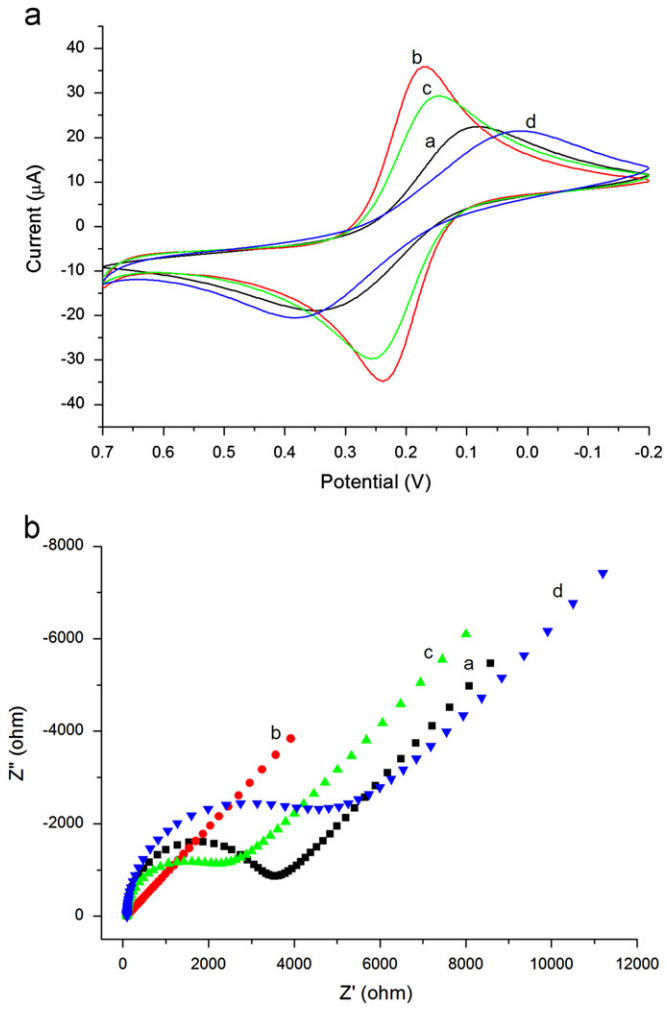


Fig. 1. (A) CV and (B) EIS characterization of the fabricated sensing interfaces using 1 mM $Fe(CN)_6^{3-/4-}$ in 1 M KCl and (a) SPE, (b) AuNPs/SPE, (c) Apt/AuNPs/SPE, and (d) MCH/Apt/AuNPs/SPE electrodes.

of $Fe(CN)_6^{3-4-}$ decreased (line c), presumably because the net negative charge provided by the phosphate groups of the immobilized aptamer sequence repelled the anionic $Fe(CN)_6^{3-/4-}$; this decrease in redox current confirmed the successful immobilization of the aptamer onto the electrode surface. Next, we treated the fabricated electrode with MCH to minimize nonspecific adsorption (i.e., to deposit MCH units onto areas of the AuNPs' surface that were not presenting aptamer molecules); this coverage of MCH minimized the exposed surface area of the AuNPs, thereby decreasing the redox current of $Fe(CN)_6^{3-/4-}$ (line d).

Fig. 1(B) displays the change in impedance of the electrode after each modification step and verifies our successful construction of the sensing interface. The trace for the bare SPE features a semicircular domain (line a). The resistance of the electrode decreased significantly after electrodeposition of the AuNPs (line b), consistent with the AuNPs having been immobilized on the SPE; the combination of high conductivity and large surface area decreased the electron transfer resistance, which increased, however, after immobilization of the anti-human IgE aptamer (line c). The net negative charge of the anti-human IgE aptamer repelled the $Fe(CN)_6^{3-/4-}$ anions and increased the resistance. The redox signal of the $Fe(CN)_6^{3-/4-}$ anions decreased greatly on the surface of the MCH/Apt/AuNPs/SPE electrode, resulting in a lower degree of electron transfer and a large semicircular trace (line d). Because the aptamer-free surface of the AuNPs was covered with MCH moieties, nonspecific adsorption in subsequent aptasensor experiments and $Fe(CN)_6^{3-/4-}$ redox processes were both inhibited at these sites. Our results obtained using EIS were consistent with those from CV, confirming the successful fabrication of the electrode device.

3.3. Amplification effect of cDNA G1 on aptasensor

We chose MB as our electrochemical redox substrate not only for its specific adsorption with guanine units of ssDNA but also for its electrochemical characteristics. The small value of ΔE_p of MB provides SWV with good sensitivity and, therefore, a better signal-to-noise (S/N) ratio for quantitation. Fig. 2 displays the MB redox signals we obtained using SWV under different sensing conditions. After immobilization of anti-human IgE aptamer, the MB molecules in the solution were strongly adsorbed by the guanine units on the exposed aptamer sequence, resulting in a significant MB redox peak appearing at −0.4 V. To prevent nonspecific adsorption of MB on the AuNPs/SPE, we treated the fabricated electrode with MCH to cover any areas of the AuNPs/SPE surface not occupied by aptamer units; this coverage of MCH also minimized the possibility of the AuNPs coming into contact with MB.

After we removed the electrode from the solution containing MB and rinsed it twice with the measuring buffer solution, the signal for the adsorbed MB still remained. The large redox current for MB on MCH/Apt/AuNPs/SPE (line a) suggests that it was the aptamer that was mainly responsible for the adsorption of the MB molecules. The redox current of MB decreased when human IgE was present (Fig. S1). The decrease in the redox current of MB could, therefore, be used for quantitation of human IgE, although the amount of guanine in the anti-human IgE aptamer sequence was limited, leading to poor sensitivity. Therefore, we designed cDNA G1 as a cDNA for competitive binding to the anti-human IgE aptamer, such that more MB molecules would bind to the guanine units on the G1 portion of cDNA G1. The redox current when MB was bound to cDNA G1 (line b) was 60% greater than that when MB was bound directly to the anti-human IgE aptamer. When we replaced cDNA G1 with cDNA, the redox current of MB decreased (line c) because MB bound weakly to the dsDNA formed from cDNA and the anti-human IgE aptamer. The significant difference in redox current between the cDNA G1/anti-human IgE aptamer and cDNA/anti-human IgE aptamer assays (lines b and c) confirms that MB adsorbed specifically to the guanine-rich section of cDNA

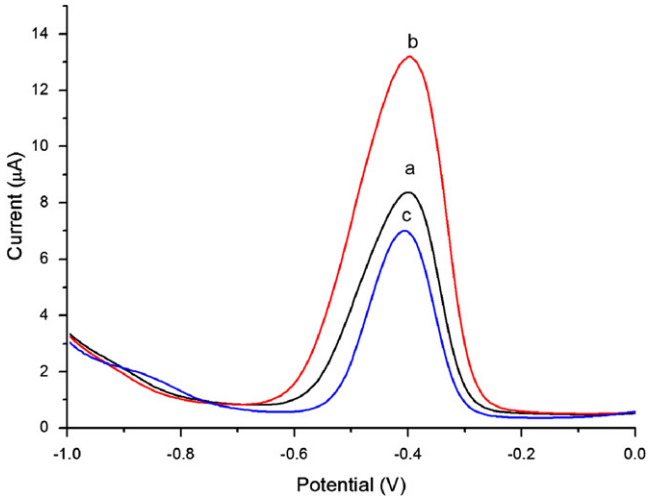


Fig. 2. Square wave voltammograms recorded using the (a) MCH/Apt/AuNPs/SPE, (b) cDNA G1/MCH/Apt/AuNPs/SPE, and (c) cDNA/MCH/Apt/AuNPs/SPE electrodes. Experimental conditions: 20 μM MB in 10 mM Tris–HCl buffer (pH 7.4) containing 2 mM KCl.

G1. Increasing the concentration of human IgE would increase the amount of human IgE bound to anti-human IgE aptamer and, therefore, decrease the binding of cDNA G1 to the aptamer. Accordingly, the redox current of MB decreased because fewer MB units were bound to cDNA G1. The guanine-rich portion of cDNA G1 could, indeed, amplify the MB redox current. Our proposed detection method, with MB attached to cDNA G1, improved the sensitivity of the aptasensor for the analysis of human IgE over that obtained using the aptamer-only electrode.

3.4. Optimization of aptasensor

We optimized the performance of the aptasensor prior to testing. To obtain the best sensitivity, we immersed the AuNPs/SPE in solutions containing different concentrations of anti-human IgE aptamer and then used SWV to measure the redox signal of MB (Fig. S2). The MB redox signal increased as we increased the concentration of the anti-human IgE aptamer from 0.1 to 1.0 μM. Increasing the concentration of the aptamer increased the cDNA G1–to–aptamer recognition and, thereby, enhanced the sensitivity of the assay. The MB redox signal decreased, however, upon increasing the concentration of the aptamer beyond 1.0 μM, presumably because fewer cDNA G1 moieties were captured by the aptamer units on the electrode. For example, steric hindrance of overpopulated anti-human IgE aptamers might have prevented cDNA G1 moieties from forming double stranded complexes with the remaining free aptamer units, thereby decreasing the MB signal. Thus, we selected 1.0 μM of the anti-human IgE aptamer as the optimal concentration for fabrication of the aptasensor.

We also evaluated the effects of adding different amounts of cDNA G1 (2 or 10 μM) on the performance of the aptasensor. The redox signal of MB for the MCH/Apt/AuNPs/SPE was satisfactory in the presence of 2 μM cDNA G1; it increased by only 7.4% when using 10 μM cDNA G1. Therefore, near-saturation occurred when employing 2 μM cDNA G1, the concentration that we used accordingly in our subsequent experiments for competitive binding with the aptamer.

3.5. Sensitivity and selectivity of aptasensor

Fig. 3 displays the SWV traces of MB that we obtained upon increasing the concentration of human IgE standard solution from 0 to 100,000 pM. The electrochemical signal of MB at −0.4 V was very intense in the absence of human IgE, due to the adsorption of MB at the guanine-rich portion of cDNA G1 bound to the aptamer. The intensity of the electrochemical signal of MB decreased upon increasing the concentration of human IgE, but it retained nearly the same peak shape and position. The relationship between the change in current of MB and the log concentration of human IgE was linear (R^2=0.996) over the range 1–100,000 pM (inset to Fig. 3). The relative standard deviation (RSD) of the aptasensor (n=3) was 10.4%, 8.8%, 2.4%, 5.2%, 0.7%, and 3.0% for the concentration of 1, 10, 100, 1,000, 10,000, and 100,000 pM IgE, respectively. The LOD of 0.16 pM appears suitable for routine analyses of human IgE and is much better than those provided by other biosensors for human IgE (Chen et al., 2009; Feng et al., 2008; Maehashi et al., 2007; Tran et al., 2011; Wang et al., 2010; Wu et al., 2009; Xu et al., 2005).

Fig. 4 displays the selectivity of our aptasensor for human IgE in the presence of interfering human IgG, thrombin, or human serum albumin (HSA). The current responses of MB from solutions (n=3) containing 100 pM human IgE spiked with 10 nM of the interfering agents were practically identical to that from the solution containing only 100 pM human IgE, revealing that the specificity of this fabricated aptasensor was adequate for analyses of human IgE.

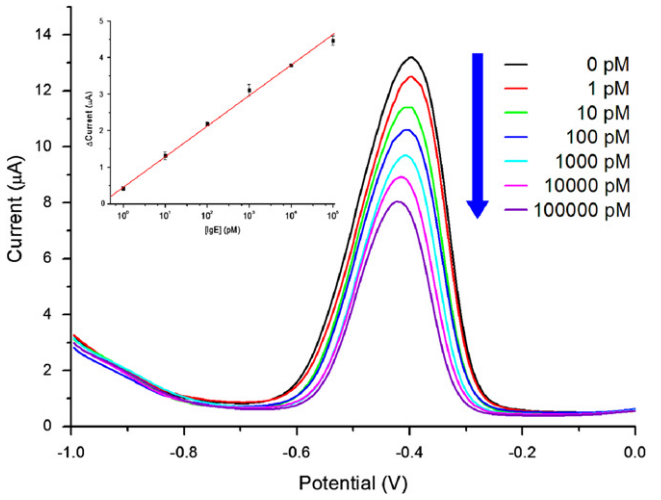


Fig. 3. Square wave voltammograms obtained when using the aptasensor to measure different concentrations of human IgE (1–100,000 pM). Inset: Calibration curve for the detection of human IgE. Error bars represent standard deviation (n=3). Experimental conditions: 2 μM cDNA G1. Other conditions were the same as those used to obtain Fig. 2.

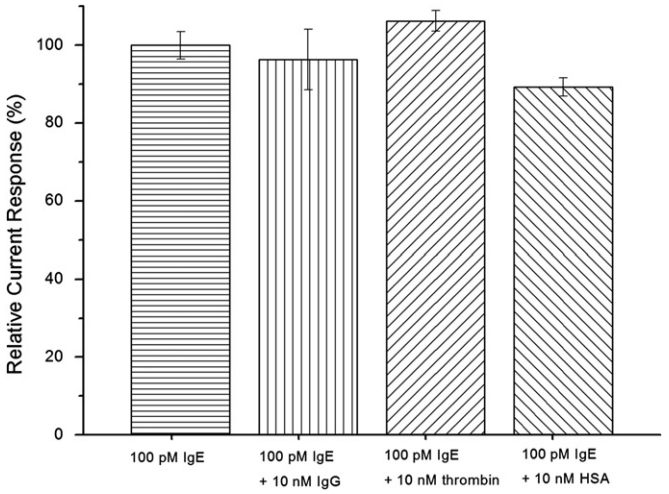


Fig. 4. Specificity of the fabricated aptasensor. Concentration of human IgE: 100 pM; concentration of human IgG, thrombin, or HSA: 10 nM. Presented data are averages from three experiments.

We also evaluated the recoveries of different concentrations of human IgE solutions spiked with 0.1 μM BSA. The recoveries (n=3) of spiked human IgE at concentrations of 10,000, 1000, and 100 pM were 104.6% (±1.81%), 108.6% (±4.31%), and 107.2% (±2.18%), respectively. These recoveries of greater than 100% arose presumably because of nonspecific absorption of BSA, which blocked the binding of cDNA G1 with the unoccupied aptamer units, thereby decreasing the current response of MB. Although the recoveries of human IgE were slightly higher than 100%, the performance of the aptasensor remained acceptable for the analysis of human IgE.

4. Conclusions

We had developed an ultrasensitive aptamer-based electrochemical biosensor that allows measurement of the concentration of human IgE. The anti-human IgE aptamer was used as a capture

probe, immobilized on an SPE modified with AuNPs. The capture of human IgE by the aptamer blocked it from binding to cDNA G1. As a result, increasing the concentration of human IgE decreased the amount of cDNA G1 that bound to the aptamer. We used the redox current of MB as the signal source because of its excellent electrochemical characteristics and its ability to bind with DNA. The binding of MB to cDNA G1 provided an amplified redox electrochemical signal, which enhanced the sensitivity of the biosensor. High linearity existed between the change in current of MB and the log concentration of human IgE over the range from 1 to 100,000 pM, with a coefficient of determination of 0.996. The LOD of the aptasensor (0.16 pM) appeared suitable for routine analyses of human IgE. The aptasensor also exhibited good selectivity and recovery. Optimizing the length of the guanine-rich section in the cDNA might further enhance the sensitivity of this aptasensor. Theoretically, this present scheme should be applicable to the sensitive detection of different analytes, merely by replacing the anti-human IgE aptamer/cDNA G1 pair with a relevant aptamer and cDNA.

Acknowledgments

This study was supported by a grant (NSC 98–2113-M-009–016-MY3) from the National Science Council and by the ATU program of the Ministry of Education, Taiwan, R.O.C.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.07.009.

References

Becker, H.C., Nordén, B., 1997. Journal of the American Chemical Society 119, 5798–5803.
Bing, T., Liu, X., Cheng, X., Cao, Z., Shangguan, D., 2010. Biosensors and Bioelectronics 25, 1487–1492.
Chen, C.-R., Liu, D.-Y., Wu, Z.-S., Luo, Q.-M., Shen, G.-L., Yu, R.-Q., 2009. Electrochemistry Communications 11, 1869–1872.
Deng, C., Chen, J., Nie, Z., Wang, M., Chu, X., Chen, X., Xiao, X., Lei, C., Yao, S., 2009. Analytical Chemistry 81, 739–745.
Du, Y., Li, B., Wei, H., Wang, Y., Wang, E., 2008. Analytical Chemistry 80, 5110–5117.
Farjami, E., Clima, L., Gothelf, K.V., Ferapontova, E.E., 2010. Analyst 135, 1443–1448.
Feng, K., Kang, Y., Zhao, J.-J., Liu, Y.-L., Jiang, J.-H., Shen, G.-L., Yu, R.-Q., 2008. Analytical Biochemistry 378, 38–42.
Fukasawa, M., Yoshida, W., Yamazaki, H., Sode, K., Ikebukuro, K., 2009. Electroanalysis 21, 1297–1302.
Gould, H.J., Sutton, B.J., Beavil, A.J., Beavil, R.L., McCloskey, N., Coker, H.A., Fear, D., Smurthwaite, L., 2003. Annual Review of Immunology 21, 579–628.
Hamaguchi, N., Ellington, A., Stanton, M., 2001. Analytical Biochemistry 294, 126–131.
Hianik, T., Wang, J., 2009. Electroanalysis 21, 1223–1235.
Iqbal, S.S., Mayo, M.W., Bruno, J.G., Bronk, B.V., Batt, C.A., Chambers, J.P., 2000. Biosensors and Bioelectronics 15, 549–578.
Jiang, Y., Fang, X., Bai, C., 2004. Analytical Chemistry 76, 5230–5235.
Kara, P., Kerman, K., Ozkan, D., Meric, B., Erdem, A., Ozkan, Z., Ozsoz, M., 2002. Electrochemistry Communications 4, 705–709.
Liao, W.-C., Ho, J.-A., 2009. Analytical Chemistry 81, 2470–2476.
Liss, M., Petersen, B., Wolf, H., Prohaska, E., 2002. Analytical Chemistry 74, 4488–4495.
Liu, J., Lu, Y., 2004. Analytical Chemistry 76, 1627–1632.
Loaiza, Ó., Campuzano, S., López-Berlanga, M., Pedrero, M., Pingarrón, J., 2005. Sensors 5, 344–363.
Maehashi, K., Katsura, T., Kerman, K., Takamura, Y., Matsumoto, K., Tamiya, E., 2007. Analytical Chemistry 79, 782–787.
Maehashi, K., Matsumoto, K., Takamura, Y., Tamiya, E., 2009. Electroanalysis 21, 1285–1290.
Peng, Q., Cao, Z., Lau, C., Kai, M., Lu, J., 2011. Analyst 136, 140–147.
Sassolas, A., Blum, L.J., Leca-Bouvier, B.D., 2009. Electroanalysis 21, 1237–1250.
Tombelli, S., Minunni, M., Mascini, M., 2005. Biosensors and Bioelectronics 20, 2424–2434.
Tran, D.T., Vermeeren, V., Grieten, L., Wenmackers, S., Wagner, P., Pollet, J., Janssen, K.P.P., Michiels, L., Lammertyn, J., 2011. Biosensors and Bioelectronics 26, 2987–2993.
Wang, J., Lv, R., Xu, J., Xu, D., Chen, H., 2008. Analytical and Bioanalytical Chemistry 390, 1059–1065.
Wang, J., Munir, A., Li, Z., Zhou, H.-S., 2010. Talanta 81, 63–67.
Winter, W.E., Hardt, N.S., Fuhrman, S., 2000. Archives of Pathology and Laboratory Medicine 124, 1382–1385.
Wu, Z.-S., Zheng, F., Shen, G.-L., Yu, R.-Q., 2009. Biomaterials 30, 2950–2955.
Xu, D.-W., Han, H.-H., He, W., Liu, Z.-H., Xu, D.-K., Liu, X.-J., 2006. Electroanalysis 18, 1815–1820.
Xu, D., Yu, X., Liu, Z., He, W., Ma, Z., 2005. Analytical Chemistry 77, 5107–5113.
Yang, W., Ozsoz, M., Hibbert, D.B., Gooding, J.J., 2002. Electroanalysis 14, 1299–1302.
Zuo, X., Xiao, Y., Plaxco, K.W., 2009. Journal of the American Chemical Society 131, 6944–6945.