Biosensors and Bioelectronics 24 (2009) 2499–2503

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Aptamer-based piezoelectric quartz crystal microbalance biosensor array for the quantification of IgE

Chunyan Yao, Yongzhi Qi, Yuhui Zhao, Yang Xiang, Qinghai Chen, Weiling Fu*

Department of Laboratory Medicine, Southwest Hospital, Third Military Medical University, Chong Qing 400038, PR China

ARTICLE INFO

Article history:
Received 21 October 2008
Received in revised form
23 December 2008
Accepted 23 December 2008
Available online 4 January 2009

Keywords:
QCM
Biosensor
Quantification
Aptamer
IgE

ABSTRACT

The aim of this study was to develop a rapid method to measure IgE in human serum by use of a direct aptamer-based biosensor based on a quartz crystal microbalance (QCM). An avidin monolayer was applied to immobilize aptamers specific for IgE on the gold surface of a quartz crystal. The frequency shifts (FS) of the QCM were measured and related to IgE concentrations. We could demonstrate that aptamers were able to detect IgE with high specificity and sensitivity in 15 min. A linear relationship existed between the FS (Hz) and the IgE concentrations from 2.5 to 200 μg/L in buffer and human serum. The regression equation was $y = 1.03x - 0.06$ for this QCM method and chemiluminescence in 50 clinical human serum samples. In addition, the aptamer receptors tolerated repeated affine layer regeneration after ligand binding and recycling of the biosensor with little loss of sensitivity. When stored for 3 weeks, the FS were all greater than 90% of those on the response at the first day. The QCM biosensor can measure IgE and offer advantages of high specificity, reusability, low detection limit, no label or sample pretreatment, and low sample requirement. The aptamer QCM biosensor was suitable for sensitive and specific protein detection, representing an innovative tool for future proteomics.

1. Introduction

An increasing number of patients are suffering from immediate-type allergic diseases (Ring et al., 2001). Rapid detection of IgE is of interest in dealing with patients afflicted with allergy-mediated disorders (Gokulrangan et al., 2005). Although it is possible to measure IgE using methods such as RIA and ELISA, these tests are expensive, time-consuming, and need a rather high volume of reagents and serum sample (Ollert et al., 2005; Brown et al., 1985; Peuravuori and Korpela, 1993). For these reasons it is highly desirable to develop a fast and economic technology for the detection of IgE. This paper explores the use of aptamers as a rapid technology for the detection of IgE.

Aptamers are functional binding species that have been selected from combinatorial oligonucleotide libraries by in vitro selection (Ellington and Szostak, 1990; Tuerk and Gold, 1990; Davis et al., 1996). Aptamers can compete with antibodies in a number of analytical applications, such as ELISA-like assays (Drolet et al., 1996), flow cytometry (Davis et al., 1998), affinity probe capillary electrophoresis (German et al., 1998), affinity chromatography (Deng et al., 2001), in high-throughput screening assays, and more generally as biosensors (Jayasena, 1999; Minunni et al., 2004). In comparison to antibodies as capture molecules in biosensors, small aptamer receptors have a number of advantages. Because of their small size, denser receptor layers can be generated. This means that for a given receptor affinity, the sensitivity of these layers can be increased (Jayasena, 1999; Minunni et al., 2004). Aptamers can be easily modified without affecting their affinity, while chemical modification of antibodies often decreases their affinity. Due to the structural instability, the immobilization of antibodies as capture molecules is causing considerable problems. It turned out that immobilization procedures influenced the biochemical activities of antibodies and even denaturation occurred in some cases. One of the obvious limitations of antibody-based biosensors is their poor capacity to regenerate the antibody surface. Although antibody-based biosensors have been demonstrated to be reusable, the loss of activity of surface-immobilized antibodies is inevitable. In contrast, aptamers can be denatured and refolded multiple times without loss of activity. That in part because the aptamer binding function is largely dependent upon simple, stable secondary structural interactions, rather than more complex, weaker tertiary structural interactions, as antibodies (Kirby et al., 2004; Liss et al., 2002). Moreover, although antibodies are readily available, many of them have high cross-reactivity with other proteins, therefore ultimately limiting their utility for detection (Mac Beath, 2002).

Biosensors, with specificity of biology reactions and high sensitivities of physical transducers, have gained attention in clinical diagnosis (Buhl et al., 2007; Yuan et al., 2004). Recently, aptamers

* Corresponding author. Tel.: +86 23 68754429; fax: +86 23 65460909.
E-mail addresses: yao_yao24@yahoo.com (C. Yao), weilingfu@yahoo.com (W. Fu).

0956-5663/$ – see front matter.
doi:10.1016/j.bios.2008.12.036

have been tested in affinity biosensors (Hianik et al., 2005; Tombelli et al., 2005; Mccauley et al., 2003). A DNA aptamer specific for human thrombin was used to detect binding of the target protein by QCM (Hianik et al., 2005). The HIV-1 Tat protein specific aptamer has been immobilized on the gold surface of piezoelectric quartz crystal to develop a QCM based biosensor (Tombelli et al., 2005). Moreover, the biosensor utilizes immobilized DNA and RNA aptamers, selected against several different protein targets, can simultaneously detect and quantify levels of individual proteins in complex biological mixtures (Mccauley et al., 2003).

This study focused on microbalances based on piezoelectric crystals, where a decrease of the resonance frequency is correlated to the mass accumulated on its surface (Huang et al., 2006). We have set up a QCM biosensor platform and successfully quantified human chorionic gonadotropin (Zhang et al., 2004) and urinary protein (Luo et al., 2006). In this paper, we constructed and evaluated a label-free, rapid QCM biosensor system. To investigate our biosensor system, we chose a well described DNA aptamer specific for IgE (MW 12 kDa, K_D = 3.6 nM) (Wiegand et al., 1996). In addition, we measured IgE concentrations in buffer and human serum with this biosensor and compared the QCM method with chemiluminescence method. Although there are some researches on IgE detection by fluorescence anisotropy, radiolabeled microarray, and quartz crystal biosensor with aptamers as bio-recognition element (Gokulrangan et al., 2005; Hianik et al., 2005; Stadtherr et al., 2005), they all deal with IgE standards but not the human serum. The comparison of the developed aptamer biosensor with these IgE detection methods is very difficult.

2. Material and methods

2.1. Reagents

Avidin, 3,3′-dithiodipropionic acid di(N-succinimidyl ester) (DSP), N,N-dimethylacetamide (DMA) were all obtained from Sigma (St. Louis, USA). Bovine serum albumin (BSA) was obtained from Roche (Indianapolis, USA). IgE was obtained from US Biological Company (MA, USA). All the other reagents were purchased from Sigma (St. Louis, USA).

The base sequence for this 37-nt, isolated from SELEX trials, has been found to be 5′-GGGGCACGTT-TATCCGTCCCTCCT AGTGGCGTGCCCC-3′ (Wiegand et al., 1996). The aptamer with the 5′-modified with biotin were custom designed and purchased from Shanghai Bioengineering Company (Shanghai, China).

2.2. Apparatus

A 10 MHz AT cut quartz crystal (13 mm × 13 mm) with gold evaporated on both sides was obtained from 26th Research Institute, Chinese Electronic Scientific and Technical Group Company (Chongqing, China). The 2 × 5 model microarray sensor was made by China Jialing Group (Chongqing, China). The frequency variations were continuously recorded using a frequency data acquisition card (Model PCL-836, Yanhua Co., Taiwan, China): the data (the resonance frequency) are displayed on the main display screen and can be read directly by a computer connected to the PCL-836 interface. The frequency shifts reported are the difference between two stable frequency values (±1 Hz). The measuring apparatus consisted of the crystals fixed inside the sensor, only one side of which was exposed to the solutions.

The electrode surface was soaked and rinsed for 10 min in cleaning solution (30% H_2O_2:98% H_2SO_4 = 1:3) and then was thoroughly washed with tri-distilled water and dried with pure nitrogen gas.

2.3. Immobilization procedure

The prepared gold surfaces were activated by injection of 10 μL of a 4 mg/mL solution of DSP in water-free DMA, and incubated at room temperature for 15 min. The surfaces were then washed 3 times with 150 μL of PBS, followed by the immediate injection of 10 μL of a 2 mg/mL solution of avidin in phosphate buffered solution (PBS), and incubated at 4 °C overnight. The 5′-biotin-labeled aptamer solutions of a concentration of 2 μmol/mL in PBS were heated to 95 °C for 3 min and chilled on ice to place the aptamer in its correct intramolecular folding. Subsequently, 10 μL of aptamer solution was added onto the surface of the crystal, and incubated at room temperature for 1 h. Any unbounded aptamers were removed by rinsing with a PBS buffer solution 3 times. To block unreacted surface groups, 10 μL of a 0.025% BSA solution was applied, and the slides were incubated at room temperature for 1 h and then rinsed by a PBS buffer solution 3 times.

2.4. Detection procedures

Ten prepared crystals were placed into the wells with the side coated with aptamer facing up to construct the 2 × 5 model array and adjust the system temperature to 37 °C. Then, 90 μL of reaction buffer was added to each cell of the sensor. The resonance frequency (F_0) was monitored until a steady baseline was obtained in 15 min during incubation. Then 10 μL of IgE standards with different concentrations (2.5–250 μg/L) were added into the wells respectively. PBS buffer was added to chamber 10 as a negative control. When the resonance frequency reached a stable value, the stable frequency was taken as F_1. The whole detection procedure was monitored, and the real-time frequency fluctuation was recorded and displayed via the software. The FS induced by aptamer-IgE ($FS = F_1 - F_0$) was detected, and the final FSs (ΔF) ΔF = FS − FSn were calculated by reading the difference between FS (sample) and FSn (negative control). In the serum sample detection procedure, 10 μL serum samples added to the crystal which did not immobilize with aptamer as the negative control. The equilibrium time for reaction was also recorded. The average value was taken by repeating the experiment 3 times.

For the study of the nonspecific binding we used human IgG, IgM, human serum albumin (HSA), and BSA. All proteins were dissolved in a PBS buffer in a concentration of 500 mg/mL.

2.5. Regeneration procedures

The regeneration solution, 0.2 mmol/L glycine/HCl (pH 2.8), 1.2 mmol/L NaOH, 30 mmol/L EDTA, 8.0 mmol/L urea and 50% formamide solution, were applied on the electrodes for 2 min to denature the aptamer and remove the IgE from it after each measurement. The obtained surfaces were then rinsed with PBS buffer 3 times. After washing with tri-distilled water the frequency reached a stable value (±1 Hz) with buffer, aptamer once again responded to the IgE and a new detection step could be performed.

2.6. Materials methodology comparison and clinical sample detection

First, we generated the calibration curves by plotting ΔF against various IgE concentrations, and the linearity was determined by serially diluting the stock IgE solutions. After the analysis procedures, the operational characteristics, including imprecision, accuracy, specificity, analytical sensitivity, and detection limit, were evaluated. The detection procedure of clinical samples was just same as that of IgE standards. All participants gave signed informed consent and the study was approved by the Ethics Boards of the Third Military Medical University. Then, the clinical samples were

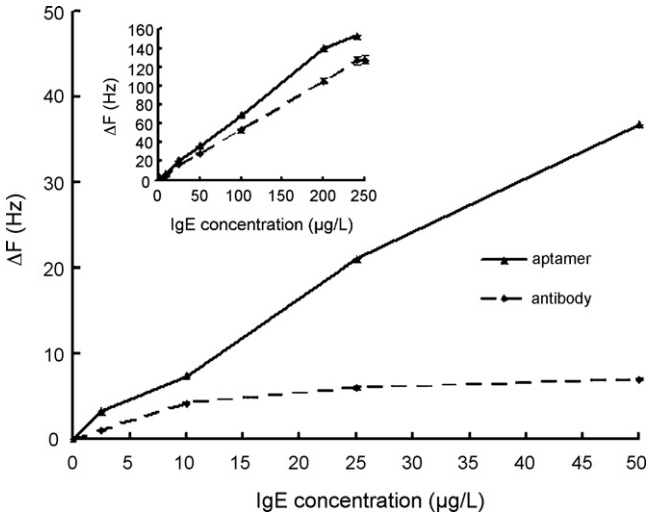

Fig. 1. The concentrations of IgE were 2.5, 10, 25, 50, 100, and 200 μg/L. The first six points were taken into account when plotting the linear relationship of FS vs IgE concentrations. Six replicates per specimen were measured.

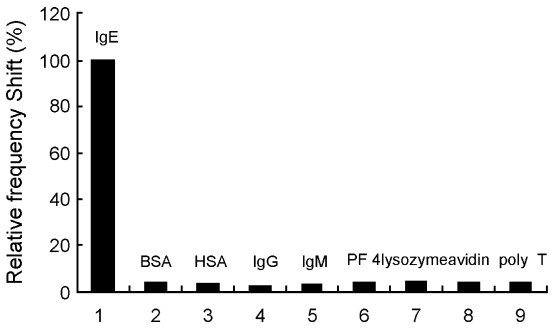

Fig. 2. Relative frequency change generated by different analyte proteins. Measurements were made under identical solution conditions. The BSA, HSA, IgG and IgM were tested at a concentration of 500 mg/L. The PF IV (platelet factor IV) and lysozyme were tested at a concentration of 1 mg/L. Experiments were also included testing 100 μg/L IgE on the avidin (the crystal modified with only avidin) and polyT (the crystal modified with a biotinylated oligonucleotide polyT). Three replicates per specimen were measured.

quantified individually with the QCM biosensor and chemiluminescence. The chemiluminescence results were obtained by the Access Immunoassay System (Beckman, USA), according to the manufacturer's protocols.

3. Results

3.1. Calibration curve for IgE

A standard curve was obtained by applying 10 μL of IgE standards at various concentrations to the aptamer-coated crystals at 37 °C. We observed linear relationships between ΔF (Hz) and the concentrations of IgE. Linear regression analysis was performed on the dose-response curves for protein concentrations >200 μg/L, however, the FS tendency increased slowly and deviated from linearity. Thus, the high concentration points should be excluded from the linear range. To obtain accurate results, sample concentrations higher than the upper limit of detection should be diluted to appropriate concentrations before detection. A linear relationship existed between the ΔF (Hz) and the concentrations of IgE in the range of 2.5–200 μg/L with a correlation coefficient of 0.996 (Fig. 1). The limit of detection (signal-to-noise ratio, >3) was measured on 20 consecutive aqueous dilutions of the negative control. The minimum detectable concentration was 2.5 μg/L. The reaction time to reach equilibrium was 15 min in buffer and human serum.

3.2. Imprecision

Imprecision data for the determination of IgE (2.5–200 μg/L) in buffer and human serum by this QCM aptamer biosensor is shown in Table 1. The mean intraassay and interassay coefficient of variation (CV) were 4.14% and 5.95%. Similarly, the intraassay and interassay CV of the chemiluminescence were 4.11% and 5.25% (Patterson et al., 1994). This result indicates that the proposed piezoelectric aptamer biosensor could provide reproducible determination of IgE. Variable surface coverage between manually produced sensing elements might account for this precision difference. Large-scale, automated fabrication of aptamer biosensors would likely yield much more uniform surface coverage and a correspondingly lower CV.

3.3. Interference of aptamer biosensor

The interfering proteins that were tested include BSA, HSA, lysozyme, IgG, IgM and platelet factor IV. These proteins were chosen to cover the range of possible interfering molecules in the detection of IgE. The relative responses of the interferences, as seen in Fig. 2, are insignificant in comparison to the specific binding response with IgE. The testing concentration of IgE was chosen as 100 μg/L since at this concentration the specific signal for IgE resulted in a lower CV (<5%). These experiments demonstrated the high specificity of the sensor with the immobilized aptamer, as a significant FS was obtained only when testing the specific protein. The values of frequency change for IgE differ significantly with that for interfering proteins according to Student's *t*-test at least with $p < 0.1$.

Interference experiments were also conducted testing IgE on the crystal modified with only avidin without the aptamer or with a biotinylated DNA oligonucleotide (polyT) immobilized on avidin. In these cases, the measurements performed with 100 μg/L IgE did not result in a measurable frequency shift. This indicates that the frequency shift due to the interaction between IgE and the immobilized aptamer was not due to the non-specific adsorption of the protein onto the coating layers

Table 1
Imprecision of the IgE determination by QCM biosensor.

Concentration	Intraassay ($n = 20$)			Interassay ($n = 20$)[a]		
IgE	Mean (μg/L)	SD (μg/L)	CV (%)	Mean (μg/L)	SD (μg/L)	CV (%)
2.5	2.82	0.14	4.96	2.27	0.19	8.37
10.0	10.17	0.34	3.34	9.61	0.58	6.04
25.0	26.03	1.17	4.49	27.56	2.13	7.73
50.0	53.18	2.63	4.94	46.94	2.64	5.62
100.0	108.21	4.76	4.40	109.19	5.02	4.60
200.0	215.98	5.81	2.69	186.99	6.28	3.36

[a] Interassay was conducted on 20 sequential days, with 10 runs/day and with three replicate tests at every IgE concentration.

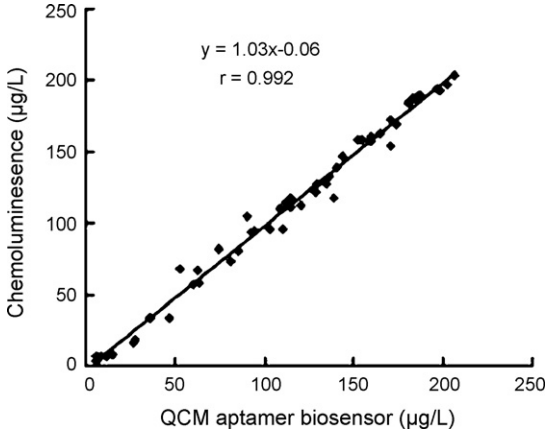

Fig. 3. Accuracy between QCM aptamer biosensor and chemiluminescence for the detection of IgE.

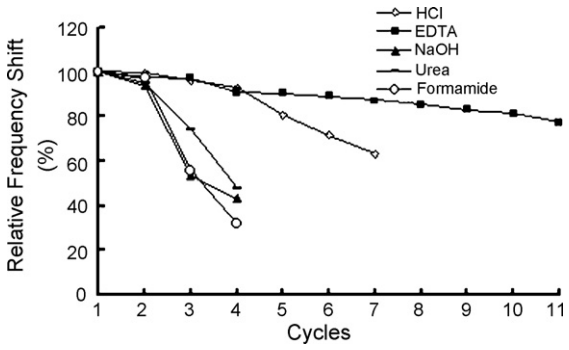

Fig. 4. Comparison of the regeneration of quartz crystals by different methods in the QCM aptamer biosensor. IgE (100 μg/L) was applied to the gold surface for detection. The regeneration reagents were 0.2 mmol/L glycine/HCl (pH 2.8) (◊), 1.2 mmol/L NaOH (▲), 30 mmol/L EDTA (■), 8.0 mmol/L urea (–), and 50% formamide (○) solution. The relative frequency shift (%) is the frequency shift measured relative to the response for the first measurement.

under the aptamer (i.e. avidin) or the biotinylated DNA oligonucleotide.

3.4. Accuracy of the QCM aptamer biosensor

We further tried IgE detection in human serum. IgE concentrations in clinical human serum samples were simultaneously measured by the QCM biosensor and chemiluminescence. Mean values by the QCM and chemiluminescence in 50 clinical human serum samples were 64.0 and 64.9 μg/L respectively, with ranges of 3–198 and 5–208 μg/L. To investigate the correlation of the QCM with the chemiluminescence, cross-comparisons for the detection results were made (Fig. 3). The correlation coefficient between QCM aptamer biosensor and chemiluminescence was 0.9924 for determination of IgE in the clinical serum samples, suggesting good consistency and clinical comparability between these two methods. More importantly, the QCM biosensor is cheaper than Access Immunoassay System and need no extra reagent added.

3.5. Recovery test of IgE in human serum

To study the feasibility of using the proposed aptamer biosensor to measure IgE levels in human serum, we added various concentrations of IgE into human serum samples. A human serum sample about 15 μg/L concentration of IgE was chosen for the test. The different concentration of IgE with low, medium, and high level was applied for the recovery test. An aliquot of 10 μL added sample was then applied onto the electrode surface of the PZ crystal and the following experiments were performed as described above. As shown in Table 2, satisfactory recovery test values were realized within the linear concentration range. A 102% recovery was achieved for the high concentration spiked samples and a 114% recovery for the 2.5 μg/L samples. The mean recovery of IgE was 105% ($n = 9$). The experimental results confirm that the proposed sensing system is applicable for IgE detection.

Table 2
Recovery test for the determination of IgE spiked in human serum.

IgE spiked (μg/L)	Final frequency (Hz)[a]	ΔF (Hz)	IgE detected (μg/L)	Recovery (%)
0.0	12	–[b]	–[b]	–[b]
2.5	15	3	2.85 ± 1.2	114
100	83	71	99.1 ± 2.1	99
180	140	128	183.1 ± 3.2	102

[a] Mean of three replicate analyses.
[b] Undetectable.

3.6. Regeneration of aptamer biosensor

The regeneration of binding surfaces is important for reusable biosensors but it often difficult to achieve. Unlike most protein biosensors, the aptamer biosensors could be denatured and reused many times on the same chip, without loss of function. Dissociating reagents were used to dissociate the bounded IgE. As shown in Fig. 4, using 30 mmol/L EDTA as regeneration reagent gave the best result. The quartz crystal could be used continuously after 10 times, and the relative frequency shifts obtained were all more than 80% of the response obtained for the first cycle. The results obtained by other regeneration solutions, 0.2 mmol/L glycine/HCl (pH 2.8), 1.2 mmol/L NaOH, 8.0 mmol/L urea, and 50% formamide solution, indicate that these reagents were not feasible perhaps due to denaturation of anti-IgE aptamer in these conditions.

3.7. Storage stability of piezoelectric aptamer biosensor

The long-term stability of the aptamer biosensor was investigated. The aptamer biosensor stored by use of 1% sodium azide solution at 4 °C was tested periodically with a 100 μg/L IgE sample. We performed five crystals for measurement continuously for 35 days. The stability was almost fully maintained at the first 7 days and then started to decline. After 3 weeks, the relative frequency shifts were about 90% compared with its initial response.

4. Discussion

Aptamers must first be immobilized on the gold surface of the quartz crystal. To sterically assist analyte binding, it is advantageous to couple the receptors to the gold surface via a linker molecule. Therefore, we chose the homobifunctional cross-linking reagent DSP, which binds to gold by a cleavable disulfide bridge and forms a peptide bond to free amino groups of lysine residues. Then, biotinylated aptamers were immobilized via DSP-bound avidin. The data presented in this report were exclusively performed with 5′-biotinylated aptamers.

The minimum detectable concentration of IgE in this study was 2.5 μg/L. While in Liss et al.'s work (Liss et al., 2002) the detection limit was 0.5 nmol/L, which equal to 95 μg/L. The difference in sensitivity from what reported by Liss et al. could be partly attributed to the bigger gold surface (a diameter of 8 mm) of the PZ crystal what they used. This usually results in a lower sensitivity. The aptamer they used was modified and had a longer sequence, that maybe another reason for the different sensitivity. This sensitivity is comparable or better than that of other reported

aptamer-based analytical methods for IgE detection, such as 46 pM with CE (German et al., 1998) and 50 pM with QCM (Liss et al., 2002), as well as the reported antibody-based ELISA (about 45 pM) (Strouse et al., 1991).

One of the greatest challenges designers of protein microarrays is nonspecific protein binding resulting in high signal backgrounds and therefore decreasing assay sensitivity. In this respect, a comparison between the signal intensities of specific IgE recognition with nonspecific binding to interfering proteins again emphasized the superiority of aptamers. The interfering proteins were chosen to cover the range of possible interfering molecules in the detection of serum sample. HSA has a much less molecular weight (MW 66,000) with respect to IgE (MW 190,000), whereas IgG is similar to IgE structurally and thus can serve as a good test protein for the aptamer binding specificity. The relatively low aptamer cross-reactivity toward BSA, HSA, and other analytes is very encouraging considering the high percentage of these serum proteins in biological samples. Lysozyme is a good control particularly in this experiment, because it has a very high basic charge (p*I* = 9.1) and is known to bind nonspecifically to nucleic acids. The results demonstrated that the contribution to the binding of the mere electrostatic interaction is reduced when considering the selected aptamer with its secondary structure. Although the binding of aptamers to nonspecific proteins could occur, it did not interfere with the highly specific binding of the aptamers to the IgE.

Aptamers can frequently be denatured and refolded multiple times without loss of activity, in part because the aptamer binding function is largely dependent upon simple, stable secondary structural interactions. Moreover, since aptamers are typically denatured and refolded during each successive round of in vitro selection, they have to some extent been "pre-evolved" for reusability. All of these considerations suggested that the aptamer chip arrays that we had produced might be reusable. In order to test the structural robustness of aptamers, aptamer-coated crystals were treated by regeneration reagents. Rinsing the surfaces with 30 mmol/L EDTA leads to dissociation of the analyte, the receptor layer being completely reconstituted simply by returning to original buffer conditions. This is because IgE aptamers need bivalent metal ions to assemble into their three-dimensional structure and unfold in the presence of EDTA. They refold very rapidly when EDTA is withdrawn and replaced by Mg^{2+}-containing buffer.

These data are the first demonstration that specific detection and quantification of proteins from serum samples is also possible with an aptamer-based biosensor. The results showed that the aptamer was able to detect IgE with high sensitivity and selectivity, even in a complex biological sample such as serum. To the best of our knowledge, this is the first report using label-free aptamers for serum protein detection.

5. Conclusions

In this study, IgE were measured by a new aptamer biosensor based on a QCM. An avidin monolayer was applied to immobilize anti-IgE aptamers on the gold surface of a quartz crystal by biotin–avidin method. The aptamer biosensor was able to detect IgE with high sensitivity and selectivity. The new aptamer biosensor array, capable of detecting IgE within 15 min, provides patients with a faster diagnosis due to a shorter detection time. Moreover, the direct biosensor system measure IgE without the need of a labeled reagent and pretreatment of samples. Considering its satisfactory precision, detection limit, linearity and accuracy, the biosensor array is suitable for IgE detection in clinical diagnostics. In conclusion, the QCM aptamer biosensor described here is a useful method that allows label-free quantitative measurement of IgE. This label-free method is simple, yet highly sensitive and selective. It has been successfully applied to the specific detection of IgE, demonstrating its potential to be a generalized method in sensitive protein assay. The label-free technique, the principal feature of the QCM biosensor array, greatly simplifies reagent preparation procedures and eliminates safety concerns associated with radioactive materials. A label-free method also avoids some essential interference induced by the fluorescence scanning procedure. The QCM aptamer biosensor array, with the advantages of accurate, fast, label-free and easy handling, provides a new possibility for further improvement in the selectivity of the clinical analytic method.

Acknowledgment

This study was supported in part by grants from the Chinese National Natural Science Foundation (30400420, 30571775), the Medical Scientific of Chinese Military (06G073).

References

Brown, C.R., Higgins, K.W., Frazer, K., Schoelz, L.K., Dyminski, J.W., Marinkovich, V.A., 1985. Clin. Chem. 31, 1500–1505.
Buhl, A., Metzger, J.H., Heegaard, N.H.H., Landenberg, P., Fleck, M., Luppa, P.B., 2007. Clin. Chem. 53, 334–341.
Davis, K.A., Abrams, B., Lin, Y., Jayasena, S.D., 1996. Nucleic Acids Res. 24, 702–706.
Davis, K.A., Lin, Y., Abrams, B., Jayasena, S.D., 1998. Nucleic Acids Res. 26, 3915–3924.
Deng, Q., German, I., Buchanan, D., Kennedy, R.T., 2001. Anal. Chem. 73, 5415–5421.
Drolet, D.W., Moon-McDermott, L., Romig, T.S., 1996. Nat. Biotechnol. 14, 1021–1025.
Ellington, A.D., Szostak, J.W., 1990. Nature 346, 818–822.
German, I., Buchanan, D.D., Kennedy, R.T., 1998. Anal. Chem. 70, 4540–4545.
Gokulrangan, G., Unruh, J.R., Holub, D.F., Ingram, B., Johnson, C.K., Wilson, G.S., 2005. Anal. Chem. 77, 1963–1970.
Hianik, T., Ostatna, V., Zajacova, Z., Stoikova, E., Evtugyn, G., 2005. Bioorg. Med. Chem. Lett. 15, 291–295.
Huang, G.S., Wang, M.T., Hong, M.Y., 2006. Analyst 131, 382–387.
Jayasena, S.D., 1999. Clin. Chem. 45, 1628–1650.
Kirby, R., Cho, E.J., Gehrke, B., Bayer, T., Park, Y.S., Neikirk, D.P., 2004. Anal. Chem. 76, 4066–4075.
Liss, M., Petersen, B., Wolf, H., Prohaska, E., 2002. Anal. Chem. 74, 4488–4495.
Luo, Y., Chen, M., Wen, Q., Zhao, M., Zhang, B., Li, X., Fu, W., 2006. Clin. Chem. 52, 2273–2280.
Mac Beath, G., 2002. Nat. Genet. 32, 526–532.
Mccauley, T.G., Hamaguchi, N., Stanton, M., 2003. Anal. Biochem. 319, 244–250.
Minunni, M., Tombelli, S., Gullotto, A., Luzi, E., Mascini, M., 2004. Biosens. Bioelectron. 20, 1149–1156.
Ollert, M., Weissenbacher, S., Rakoski, J., Ring, J., 2005. Clin. Chem. 51, 1241–1249.
Patterson, W., Werness, P., Payne, W.J., Matsson, P., Leflar, C., Melander, T., 1994. Clin. Chem. 40, 2042–2045.
Peuravuori, H., Korpela, T., 1993. Clin. Chem. 39, 846–851.
Ring, J., Krämer, U., Schäfer, T., Behrendt, H., 2001. Curr. Opin. Immunol. 13, 701–708.
Stadtherr, K., Wolf, H., Lindner, P., 2005. Anal. Chem. 77, 3437–3443.
Strouse, R.J., Anderson, D.W., Argentieri, D.C., 1991. J. Immunoassay 12, 113–124.
Tombelli, S., Minunni, M., Luzi, E., Mascini, M., 2005. Bioelectrochemistry 67, 135–141.
Tuerk, C., Gold, L., 1990. Science 249, 505–510.
Wiegand, T.W., Williams, P.B., Dreskin, S.C., Jouvin, M.H., Kinet, J.P., Tasset, D., 1996. J. Immunol. 157, 221–230.
Yuan, B.F., Hao, Y.H., Tan, Z., 2004. Clin. Chem. 50, 1057–1060.
Zhang, B., Mao, Q., Zhang, X., Jiang, T., Chen, M., Yu, F., Fu, W., 2004. Biosens. Bioelectron. 19, 711–720.