

Development of a Label Free IGE Sensitive Aptasensor Based on Electrochemical Impedance Spectrometry

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Abstract: A sensitive aptamer based electrochemical biosensor to detect human immunoglobulin E (IgE) is presented in this study. 5' Biotin labeled 45 mer DNA aptamer sequence was immobilized onto streptavidin coated graphite surfaces. Interaction between human IgE and DNA aptamer was monitored by Electrochemical Impedance Spectrometry (EIS) in a 0.48nM detection limit of IgE. EIS analyses are based on electron transfer resistance (R_{ct}) in the presence of 5mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$.

Keywords: Aptamer, IgE, biosensor, electrochemical impedance spectrometry (EIS).

1. INTRODUCTION

Aptamers are short single-stranded DNA or RNA sequences which have high specific affinity in binding protein targets [1]. Aptamer based studies represent a novel modern and a strategic approach in bioanalytical analysis for specific protein detection [2, 3]. Due to their high stability, chemical simplicity and easy applicability, they can be used as alternative captures to antibodies [4, 5]. Thereby, aptamer applications hold a great promise for biosensing of protein, and for developing protein arrays [6, 7].

In recent years, there has been a great interest in developing aptamer sensors, thereby different strategies have been developed for aptamer based protein screening. Affinity biosensors have a great promise in aptamer sensing analysis due to their, high sensitivity, selectivity, simple instrumentation, portable and cost effectiveness [8]. Thus, a lot of aptamer based sensing interfaces have been developed including optical [9-11], piezoelectrical [12] transduction, fluorescence [13, 14] and atomic force microscopy [15].

Current studies based on electrochemical methods represent a new approach for molecular recognition of aptamer – protein interaction. Various formats have reported that, a single-stranded aptamer sequence with an electroactive label, such as methylene blue or ferrocene, immobilized onto electrode surface, protein binding and electrochemical monitoring were performed in same solution [16-19].

In recent studies, electrochemical impedance spectrometric transduction of aptamer based protein analysis have shown a great prospect for label -free detections [20-22]. Electrochemical impedance spectroscopy (EIS) has been proven as one of the most powerful analytical tools for diagnostic analysis based on interfacial investigations. EIS measures the response of an electrochemical system to an applied oscillating potential as a function of the frequency. Impedimetric techniques have been developed to characterize

the fabrication of the biosensors and to monitor the catalyzed reactions of biomolecules, such as enzymes, proteins, nucleic acids, whole cells and antibodies [23, 24].

Here in, we describe a label-free bioelectronic detection of aptamer – IgE interaction based on EIS technique. The 5'biotinylated aptamer sequence was immobilized onto streptavidin coated disposable graphite electrode (DGE) surfaces. The binding of IgE to aptamer sequence was monitored by EIS transduction of the R_{ct} in the presence of 5mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$. This article gives an overview of alternative electrochemical biosensors for the detection of DNA- protein interactions.

2. MATERIALS AND METHODS

2.1. Apparatus

Streptavidin, N-hydroxysulfsuccinimide (NHS), [n-(3-dimethylamino)propyl]-N-ethylcarbodiimide (EDC) and Trizma hydrochlorides were purchased from Sigma-Aldrich Chemical Company (Germany), and tri-Natrium citrate were purchased from Merck (Germany). Other chemicals were of analytical reagent grade and supplied Merck and Sigma. Ultrapure distilled water was used in all solutions, and all the experiments were performed at room temperature.

DNA aptamer that selective bind human IgE with the 5' modified with Biotin was purchased from sequence was purchased from TIB MOLBIOL – Germany. Human IgE clone HE 1 was purchased from Bioparto Diagnostics – UK. The aptamer sequence has a following base composition [10-5]:

5' Biotin - GCG CGG GGC ACG TTT ATC CGT CCC TCC TAG TGG CGT GCC CCG CGC 3'.

2.2. Electrochemical Apparatus

Electrochemical impedance spectrometry (EIS) were performed by using AUTOLAB PGSTAT-10 –FRA, Differential pulse voltammetry (DPV) experiments were performed by using an AUTOLAB PGSTAT-30 electrochemical analysis system (Eco Chemie -The Netherlands). The three electrode system consisted of a DGE

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as working electrode, an Ag/AgCl reference electrode, and a platinum wire auxiliary electrode.

2.3. Aptasensor Preparation

2.3.1. Electrode Pretreatment

The DGE surfaces were chemically modified by exposure to 0.5 M phosphate buffer containing 5mmol/L EDC and 8mmol/L NHS used for streptavidin coupling.

2.3.2. Streptavidin Biomodification

Prior to aptamer immobilization, DGE's were modified with streptavidin via carbodiimide chemistry. DGE's were immersed into 0.5 M phosphate buffer containing 1 μ g/mL streptavidin (pH = 7.4) for 1 hour.

2.3.3. Aptamer Biomodification

5' biotinylated 45 – mer IgE sensitive aptamer was immobilized on to streptavidin coated DGE surfaces by immersing the modified electrodes in to 0.7 μ M concentration of the aptamer solution for 1 hour. % 1 BSA solution prepared in phosphate buffer was used as a blocking agent.

2.4. IgE Accumulation

Aptamer biomodified sensors were incubated with PBS buffer containing 1 μ g/mL concentration of IgE for 1 hour. 1 μ g/mL concentration of BSA is also used as non – specific protein

2.5. Detection of IgE by EIS Spectroscopy

EIS used for IgE detection. Modified DGE's were measured in a PBS (pH 7.4) in the presence of 0.5mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at +0.24 V. A frequency range from 10 kHz to 50 mHz and an AC amplitude of 10mV were applied. The impedance data was fitted to an equivalent circuit.

3. RESULTS AND DISCUSSION

An impedimetric system was proposed for direct electrochemical detection of IgE *via* aptamer – protein interaction. Disposable graphite electrodes were used as analytical surface. 5'biotinylated aptamer sequence was immobilized on to streptavidine coated surfaces and IgE detection was accomplished by EIS transduction of aptamer – protein interaction.

Fig. (1) represents the Impedance Spectra of 5mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ at +0.24 V at DGE electrode surface before and after aptamer – protein interaction. Incubation with aptamer sequence (Fig. 1b) resulted in an increase in the semicircle diameter as compared with blank electrode responses (Fig. 1a), incubation with aptamer – protein (IgE) complex (Fig. 1d) resulted in significant admittance increases as compared with only aptamer modified electrode responses, also incubation with BSA solution as a control experiment of non-specific bounding resulted (Fig. 1c) in a decrease in the semicircle diameter as compared with aptamer-IgE complex and also bare electrode responses (Fig. 1), as expected.

Experiments reproduced several times and repeatability of the responses was in a fair condition.

The AC imaginary faradaic admittance response of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (5×10^{-6} M, 1:1), was measured to determine the peak potential of the $[\text{Fe}(\text{CN})_6]^{3-/4-}$.

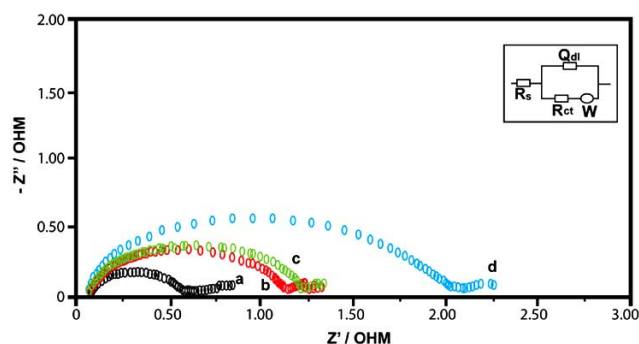


Fig. (1). Impedance Spectra of chemically pretreated (a) bare electrode, (b) after aptamer immobilization, (c) after interaction with non-specific BSA and (d) after interaction with IgE targets, at graphite electrode surface. Measurements performed in a 0.05 M phosphate buffer (pH 7.4) in the presence of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (5×10^{-6} M, 1:1) at +0.24 V.

Fig. (2) represents the histammograms of Rct values obtained after electrode pretreatment with EDC &NHS solutions (A), streptavidin biomodification of pretreated DGE's (B), aptamer biomodification at streptavidin coated surfaces (C), IgE interaction at aptamer modified surfaces (D) and after BSA interaction at aptamer modified surfaces (E) as control experiment of non specific bounding. It observed an increase in EIS responses after streptavidin binding at pretreated DGE surfaces. After 5'biotin labeled aptamer sequence biomodification at streptavidin coated DGE's a significant increase was observed in responses, compared with before modification. The interaction between IgE and aptamer sequences was observed by increasing the EIS response due to comparison of the responses before interaction. Control experiments were performed by using BSA interaction with aptamer sequence such as; after BSA accumulation it was observed that no significant change in the response comparing to aptamer modified DGE's. Thus here we demonstrate the detection efficiency and the selectivity of the designed aptasensor. RSD values are given as error bars.

To qualitatively understand the human IgE protein concentration at aptamer modified surface, the magnitudes of Rct values after interaction with aptamer sequence, were recorded. The calibration plot due to EIS responses is shown in Fig. (3). Various concentrations of IgE solutions were trialed to optimize the sensitivity. Elected IgE concentration values were from 0 μ g/mL up to 10 μ g/mL RSD values are given as error bars. Both full saturation degree, optimum value of the responses and low error rate, were obtained with 1 μ g/mL concentration of human IgE.

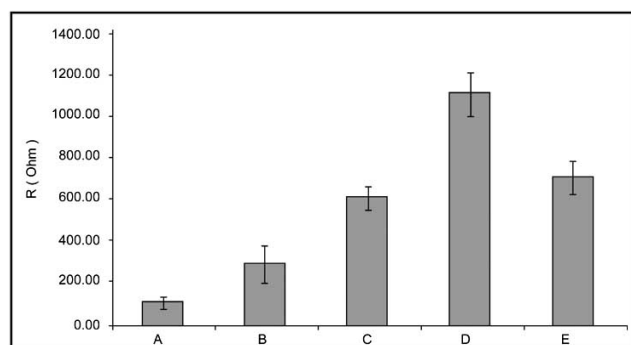


Fig. (2). EIS transduction of Rct values obtained from (A) chemically pretreated bare electrodes, (B) streptavidin modified bare electrodes, (C) aptamer biomodification on streptavidin coated electrodes, (D) human IgE interaction with aptamer modified electrodes and (E) BSA interaction as control experiment, at aptamer modified electrodes.

The detection limit estimated from $S/N = 3$ correspond to 0.48 nM at IgE sensitive aptamer modified DGE's.

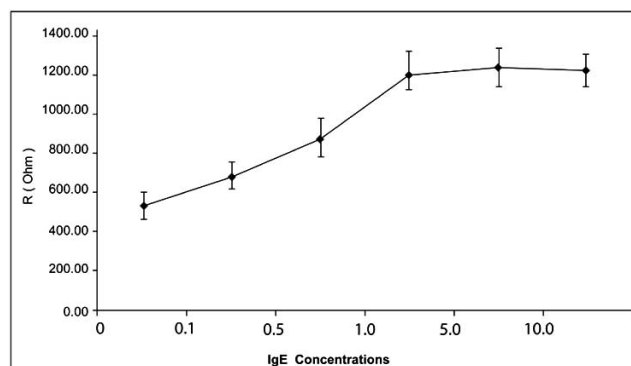


Fig. (3). Calibration plot for dependence of Rct values at upon increasing the target IgE concentration from 0 µg/mL to 10 µg/mL at aptamer modified DGE surfaces.

4. CONCLUSION

Here, we have described a label free impedimetric aptasensor that provides sensitive detection of human IgE protein. For the detection 5' Biotin labeled aptamer sequence was immobilized on to streptavidin coated disposable graphite electrodes and human IgE was interacted. The detection, was accomplished by electrochemical impedance spectrometric transduction the Rct values in the presence of $[\text{Fe}(\text{CN})_6]^{3-/4-}$. The selectivity of aptasensor was evaluated by using the EIS response differences between aptamer- IgE and aptamer -BSA interaction. Depend on detection limit, such as 0.48 nM; even it is incomparable with previous studies it was shown that the designed IgE sensitive aptasensor is capable of label free; low-cost, fast and reliable electrochemical detection. Furthermore by using different aptamer sequences, more types of proteins can be detected.

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REFERENCES

- [1] Zhang, Y.L.; Huang, Y.; Jiang, J.H.; Shen, G.L.; Yu, R.Q. Electrochemical aptasensor based on proximity-dependent surface hybridization assay for single-step, reusable, sensitive protein detection. *J. Am. Chem. Soc.*, **2007**, *129*, 15448-15449.
- [2] Tombelli, S.; Minunni, M.; Luzzi, E.; Mascini, M. Aptamer-based biosensors for the detection of HIV-1 Tat protein. *Bioelectrochemistry*, **2005**, *67*, 135-141.
- [3] Bini, A.; Minunni, M.; Tombelli, S.; Centi, S.; Mascini, M. Analytical Performances of Aptamer-Based Sensing for Thrombin Detection. *Anal. Chem.*, **2007**, *79*(7), 3016-3019.
- [4] Radi, A.E.; Sanches, J.L.A.; Baldrich, E.; O'Sullivan, C.K. Reagentless, reusable, ultrasensitive electrochemical molecular beacon aptasensor. *J. Am. Chem. Soc.*, **2006**, *128*, 117-124.
- [5] Numnuam, A.; Torres, K.; Xiang, Y.; Bash, R.; Thavarungkul, P.; Kanatharana, P.; Pretsch, E.; Wang, J.; Bakker, E. Aptamer-based potentiometric measurements of proteins using ion-selective microelectrodes. *Anal. Chem.*, **2008**, *80*, 707-712.
- [6] Baker, B.R.; Lai, R.Y.; Wood, M.S.; Doctor, E.H.; Heeger, A.J.; Plaxo, K.W. 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*, 3138-3139.
- [7] Han, K.; Chen, L.; Lin, Z.; Li, G. Target induced dissociation (TID) strategy for the development of electrochemical aptamer-based biosensor. *Electrochem. Commun.*, **2009**, *11*, 157-160.
- [8] Du, Y.; Li, B.; Wei, H.; Wang, Y.; Wang, E.; Multifunctional label-free electrochemical biosensor based on an integrated aptamer. *Anal. Chem.*, **2008**, *80*, 5110-5117.
- [9] McCauley, T.G.; Hamaguchi, N.; Stanton, M. Aptamer-based biosensor arrays for detection and quantification of biological macromolecules. *Anal. Biochem.*, **2003**, *319*, 244-250.
- [10] Minunni, M.; Tombelli, S.; Fonti, J.; Spiriti, M.; Mascini, M.; Bogani, P.; Buiatti, M. Detection of fragmented genomic DNA by PCR-free piezoelectric sensing using a denaturation approach. *J. Am. Chem. Soc.*, **2005**, *127*, 7966-7967.
- [11] Pavlov, V.; Shlyahovsky, B.; Wilner, I. Fluorescence detection of DNA by the catalytic activation of an aptamer/thrombin complex. *J. Am. Chem. Soc.*, **2005**, *127*, 6522-6523.
- [12] Hianik, T.; Ostatna, V.; Zajacova, Z.; Stoikova, E.; Evtugyn, G. Detection of aptamer-protein interactions using QCM and electrochemical indicator methods. *Bioorg. Med. Chem.*, **2005**, *15*, 291-295.
- [13] Jiang, Y.X.; Fang, X.H.; Bai, C.L. Signaling aptamer/protein binding by a molecular light switch complex. *Anal. Chem.*, **2004**, *76*, 5230-5235.
- [14] Wang, J.; Jiang, J.X.; Zhou, Z.S.; Fang, X.H. Aptamer-based ATP assay using a luminescent light switching complex. *Anal. Chem.*, **2005**, *77*, 3542-3546.
- [15] Basnar, B.; Elnathan, R.; Wilner, I. Following aptamer-thrombin binding by force measurements. *Anal. Chem.*, **2006**, *78*, 3638-3642.
- [16] Radi, A.E.; O'Sullivan, C.K. Aptamer conformational switch as sensitive electrochemical biosensor for potassium ion recognition. *Chem. Commun. (Camb.)*, **2006**, *32*, 3432-3434.
- [17] Tan, S.E. Q.; Wivanius, R.; Toh, C.S. Heterogeneous and homogeneous aptamer-based electrochemical sensors for thrombin. *Electroanalysis*, **2009**, *21*, 749-754.
- [18] Ferapontova, E.; Gothelf, K.V. Optimization of the electrochemical RNA-aptamer based biosensor for theophylline by using a methylene blue redox label. *Electroanalysis*, **2009**, *21*, 1261-1266.
- [19] Evtugyn, G.; Porfireva, A.; Ivanov, A.; Kononova, O.; Hianik, T. Molecularly imprinted polymerized methylene green as a platform for electrochemical sensing of aptamer-thrombin interactions. *Electroanalysis*, **2009**, *21*, 1272-1277.
- [20] Rodrigez, C.M.; Kawde, A.N.; Wang, J. Aptamer biosensor for label-free impedance spectroscopy detection of proteins based on recognition-induced switching of the surface charge. *Chem. Commun. (Camb.)*, **2005**, *34*, 4267-4269.
- [21] Deng, C.; Chen, J.; Nie, Z.; Wang, M.; Chu, X.; Chen, X.; Xiao, X.; Lei, C.; Yao, S. Impedimetric aptasensor with femtomolar sensitivity based on the enlargement of surface-charged gold nanoparticles. *Anal. Chem.*, **2009**, *81*, 739-745.
- [22] Sharon, E.; Freeman, R.; Tel-Vered, R.; Wilner, I. Impedimetric or ion-sensitive field-effect transistor (ISFET) aptasensors based on the self-assembly of an nanoparticle-functionalized supramolecular aptamer nanostructures. *Electroanalysis*, **2009**, *21*, 1291-1296.

[23] Steichen, M.; Herman, C.B. Electrochemical detection of the immobilization and hybridization of unlabeled linear and hairpin DNA on gold. *Electrochem.Comm.*, **2005**, 7, 416-420.

[24] Steichen, M.; Decrem, Y.; Godfroid, E.; Herman, C.B. Electrochemical DNA hybridization detection using peptide nucleic acids and $[\text{Ru}(\text{NH}_3)_6]^{3+}$ on gold electrodes. *Biosens. Bioelectron.*, **2007**, 22, 2237-2243.

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