Development of an Electrochemical Aptasensor for Detection of Ochratoxin A

Okratoksin A Tayinine Yönelik Elektrokimyasal Aptasensör Geliştirilmesi

Research Article

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ABSTRACT

A sensitive electrochemical aptasensor for label free detection of Ochratoxin A (OTA) is presented in this study. 5'thiohexyl (SH- $(CH2)_6$) linked aptamer sequence was immobilized onto disposable screen printed gold electrode surfaces (AuSCPE) via thiol bounding. Interaction between OTA and DNA aptamer was monitored by Electrochemical Impedance Spectrometry (EIS) in the presence of 5mM $[Fe(CN)_6]^{3-/4-}$ based on electron transfer resistance (Rct). And a limit of detection was found to be 0.375 nM.

Key Words
Aptasensor, ochratoxin A, electrochemical impedance spectrometry (EIS).

ÖZ

Çalışmada Okratoksin A'nın (OTA) işaretsiz bir yöntemle tayinine yönelik elektrokimyasal aptasensör tasarımı gerçekleştirilmiştir. 5' ucundan tiyohekzil işaretli (SH-$(CH_2)_6$) aptamer dizileri tek kullanımlık perde baskılı altın elektrot (AuSCPE) yüzeylerine tiyol grubu ile bağlanmıştır. OTA ve DNA aptamer dizisi arasındaki bağlanma Elektrokimyasal Empedans Spektrometri tekniği (EIS) ile 5mM $[Fe(CN)_6]^{3-/4-}$ çözeltisi varlığında elektron aktarımına dayalı olarak tayin edilmiş ve tayin sınırı da 0.375 nM olarak saptanmıştır.

Anahtar Kelimeler
Aptasensor, ochratoxin A, electrochemical impedance spectrometry (EIS).

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INTRODUCTION

Ochratoxin A (OTA) is a mycotoxin which is an hazardous contaminant exist in a great number of agricultural products such as cereals, coffee beans, dried fruits, cocoa, nuts, beer, and wine, causing many problems in food safety [1,2]. Furthermore, OTA has been reported in the literature as a nephrotoxic, teratogenic, immunotoxic agent, class 2B human carcinogen by the International Agency for Research on Cancer [3,4].

Currently, the most widely applied methods for OTA detection are based on chromatographic and immunoassay techniques [5-7]. But these techniques are high cost, long processing times, and the need of specially trained operators. Detection methodologies based on immunoassays have some advantages of simplicity and reliability [8,9]. However, antibodies may also have some problems about stability and modification in the storage and application conditions.

In recent years, aptamer based detection techniques hold great promise in replacing antibodies. These synthetic nucleic acid sequences act as antibodies in binding biological targets due to their low cost production and modification, high stability, specificity for a wide range of targets and their thermal and chemical stability, [10,11]. Therefore aptamer based biosensors (aptasensors) for the detection of several toxins such as OTA are increasing in food analysis. OTA sensitive aptasensors which have different transducers including optical, piezoelectrical, electrochemical have been reported in the literature [12 -16]. Among these techniques, electrochemical methods provide label free, rapid, sensitive and selective detection [17-21].

In this work, the development of a label free OTA aptasensor based on electrochemical impedance spectrometry was performed at AuSCPE surfaces. 5'thiolated aptamer sequence was modified onto sensor surfaces via thiol links and mercaptohexanol was used for surface blockage, the detection of interaction between aptamer –OTA was performed by EIS transduction of the Rct in the presence of 5mM $[Fe(CN)_6]^{3-/4-}$. This article gives an overview for alternative electrochemical biosensors for the detection of aptamer- mycotoxin interactions.

MATERIALS and METHODS

Apparatus and electrodes

Electrochemical impedance spectrometry (EIS) measurements were performed by using AUTOLAB PGSTAT-30 –FRA, (Eco Chemie, The Netherlands). Screen printed gold electrode (AuSCPE) containing the three electrode system as working electrode, an Ag/AgCl reference electrode, and auxiliary electrode were purchased from Dropsens (Spain).

Reagents and Solutions

Mercaptohexanol and ochcratoxin A was purchased from Sigma Aldrich. Other chemicals were of analytical reagent grade and supplied Merck and Sigma. Ultrapure distilled water was used in all solutions. And all experiments were performed at room temperature.

DNA aptamer sequence that selective to ochratoxin A with the 5' modified with thiohexyl was purchased from TIB MOLBIOL – Germany. The aptamer sequence has a following base composition [22]:

5' SH (CH2)6 – GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA 3'

Methods

Sensor Surface Biomodification: 5' thiolated OTA sensitive aptamer was immobilized on to AuSCPE surfaces by immersing the 20 μg/mL concentration of the aptamer solution for 3 hour. And same concentration of mercaptıhexanol solution was used as a blocking agent for 1 hour.
Aptamer/OTA complex formation: OTA solution at a concentration of 30 μg/mL was accumulated onto the aptamer modified AuSCPE's by immersing during 1 h the sensors into a OTA solution prepared in PBS buffer. Blank assays were performed by following the same protocol but using same concentration of bovine serum albumin (BSA) instead of OTA.

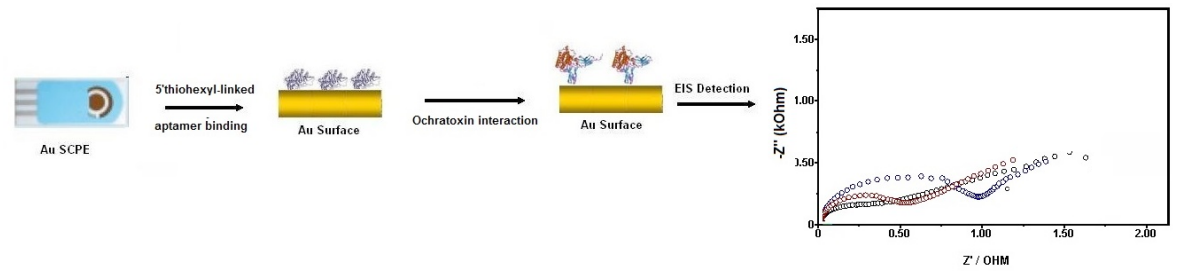

Figure 1. Schematic illustration of general study procedure.

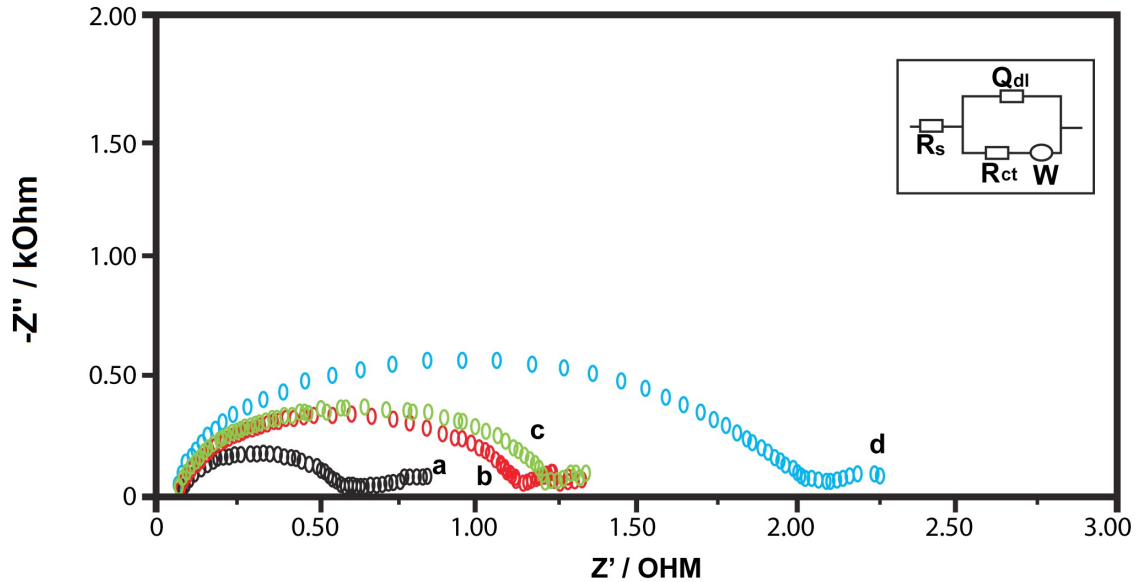

Figure 2. Rct values obtained in the prsence of 5mM $Fe(CN)_6]^{3-/4-}$ at (a) bare, (b) aptamer modified, (c) aptamer –BSA interaction, (d) aptamer –OTA interaction AuSCPE surfaces.

Electrochemical Impedance Spectrometry (EIS) Based Detection: The AuSPCEs were immersed into a 5 mM $[Fe(CN_6)]^{3-/4-}$ solution prepared in PBS 50 mM pH 7.4 solution and a potential of +0.24 V was applied. A frequency range from 10 kHz to 50 mHz and an AC amplitude of 10 mV were fixed. The impedance data was fitted to an equivalent circuit, and the value of the resistance to charge transfer (Rct) was chosen as the analytical signal.

RESULTS and DISCUSSION

A label-free impedimetric aptasensor device has been developed for the direct detection of OTA by using AuSCPEs as electrochemical transducers. 5′ thiohexyl linked aptamer sequence was immobilized onto the AuSPCE surfaces via –SH links, OTA detection was accomplished by EIS transduction of aptamer – target molecule interaction. A general scheme of the experimental procedure is shown in Figure 1.

Figure 2 represents the Impedance Spectra of 5 mM $Fe(CN)_6]^{3-/4-}$ at +0.24 V before and after aptamer interactions and bare AuSCPEs. Incubation with aptamer biomodification (Fig. 2b) resulted in increase of semicircle diameter as compared with blank electrode responses (Figure 2a), incubation with aptamer – OTA complex (Figure 2d) resulted 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 (Figure 2c) in decreases of semicircle diameter as compared with aptamer –OTA complex as expected. Experiments reproduced several times and repeatability of the responses was in a fair condition.

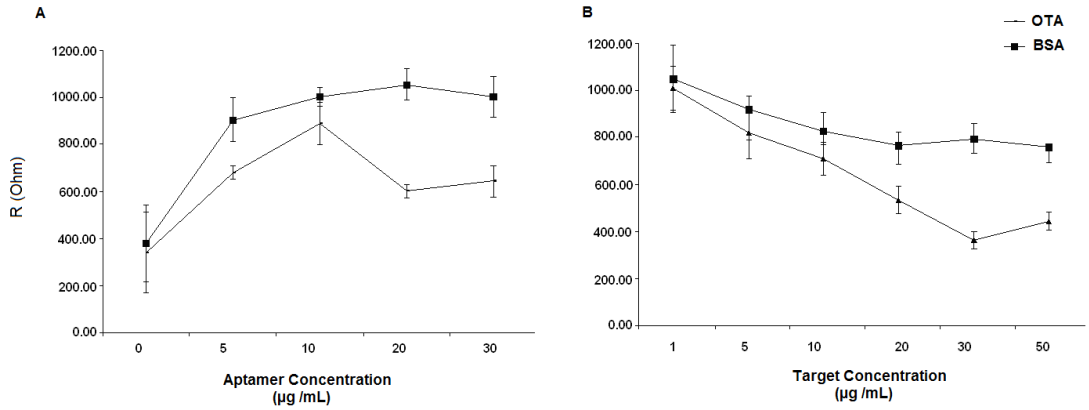

Figure 3. Rct values in the presence of 5mM $[Fe(CN)_6]^{3-/4-}$ by increasing the concentrations of A) aptamer B) target at aptamer modified surfaces after interaction with OTA target and nonspecific BSA molecules.

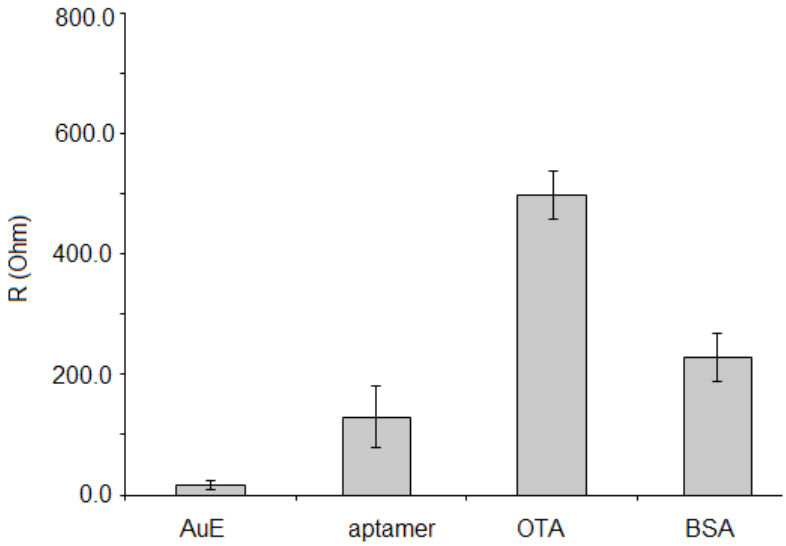

Figure 4. Histammograms obtained from Rct values in the presence of 5 mM $[Fe(CN)_6]^{3-/4-}$ following the experimental procedure detailed in section 2.3 at AuSCPE surfaces under optimum conditions.

The effects of aptamer and target concentrations on the analytical signal (Rct) for the OTA sensitive aptamer modified AuSPCEs were evaluated. Figure 3 represents the impedimetric spectra obtained for increased concentrations of (A) aptamer sequence from 0.0 to 30.0 µg/mLm and (B) target molecule from 1.0 to 50 µg/mL.

Optimum value of the responses, maximum differentiation between nonspecific BSA and low error rates were obtained with 20 µg/mL concentration of aptamer sequnece and 30 µg/mL of target. From this study, a limit of detection was estimated as 0.375 nM.

Finally, under optimum conditions selectivity assays were performed in Figure 4. When only the aptamer was immobilized onto the surface an increase in the Rct values was observed. After interaction with OTA as target molecule highly increase was occurred in Rct response. However, a significant decrease was observed after interaction with nonspecific BSA target at aptamer modified AuSCPE's in comparision with OTA responses demonstrating the selectivity of the designed aptasensor. Thus, here we demonstrate the detection efficiency and the selectivity of the designed aptasensor. RSD values are given as error bars.

CONCLUSION

Here we described a label free impidimetric aptasensor for sensitive detection of ochratoxin A molecule. The selectivity of aptasensor was evaluated by using the EIS response differences between aptamer-OTA and aptamer–BSA interaction. The OTA detection limit of 0.375nM shows that the designed OTA sensitive aptasensor is capable of label free; low-cost, fast and reliable electrochemical detection. Furthermore, by using different aptamer sequences, more types of toxins can be detected in food safety.

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