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COMMUNICATION

Label-free aptamer-based electrochemical impedance biosensor for 17β-estradiol

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A novel aptamer-based label-free electrochemical impedance spectroscopy biosensor for 17β-estradiol has been fabricated. The aptamers were firstly immobilized on the gold electrode through Au–S interaction; the aptamer probe was then bound with the addition of 17β-estradiol to form the estradiol/aptamer complex on the electrode surface. This leads to a significantly larger interfacial electron transfer resistance than that without the addition of 17β-estradiol. The change in the resistance had a linear relationship with 17β-estradiol concentration in the range of 1.0×10^{-8} to 1.0×10^{-11} mol L^{-1}, with a detection limit of 2.0×10^{-12} mol L^{-1}. The biosensor showed high selectivity to 17β-estradiol and good stability. The designed biosensor has been applied to detect 17β-estradiol in human urine with satisfactory results.

1. Introduction

Endocrine disrupting compounds (EDCs) are a class of exogenous estrogen substances obtained from man-made or natural sources, having the function similar to endogenous estrogen.[1–3] They could disrupt the endocrine system, further causing adverse effects on the reproduction, growth and development of the body, and endanger the health of offspring.[4,5] 17β-Estradiol is one of the most potential environmental endogenous estrogens: when one is chronically exposed to 17β-estradiol, it may cause drastic problems to human health.[6] Therefore, there is a need to develop a sensitive method for 17β-estradiol detection.

Conventional methods such as HPLC[7] and GC-MS[8] are typical technologies for the sensitive detection of these toxic endocrine disrupting chemicals, but they require expensive equipments and complicated performance procedures. Recently, biosensors for 17β-estradiol determination have received great attention due to their remarkable advantages of inexpensive instrumentation, high specificity and fast response.

Seifert *et al.*[9] proposed an enzyme linked receptor assay (ELRA) for 17β-estradiol determination based on the human estrogen receptor for binding estrogenic substances in environmental samples. It yielded a detection limit of up to 0.1 mg mL^{-1}. Although the human estrogen-receptor (hER) has been applied in biosensors frequently, hER has a high affinity with other xenoendocrines; so, the specificity of the biosensor is not satisfactory. Wei's group[1] built a Chemiluminescence Enzyme Immunoassay that can be applied to detect 17β-estradiol in river water and human urine samples; since antibodies must be produced by the body's immune system and cannot be reproducibly synthesized, this method is not economical.

After the pioneering work by the groups of Ellington[12] and Gold[13] who selected aptamers for specificity binding of macromolecules or small molecules, many efforts have been devoted to design aptamer-based biosensors based on different analytical techniques, such as fluorescence,[14–16] quartz crystal microbalance[17,18] and electrochemistry.[19–23] An aptamer-based 'signal off' electrochemical biosensor has been developed to detect 17β-estradiol,[10] which combines the high sensitivity of electrochemistry and unique properties of the aptamer; the aptamer was modified on the electrode surface, and then the targets bound with the aptamer, which block the electron flow of the redox reaction on the electrode surface, resulting in a decreased current. The target can suppress up to 100% of the original signal, which influences the detection limit. Therefore, it is very significant to develop a 'signal on' biosensor for 17β-estradiol. Mostly, aptamer-based biosensors need the modification of a signal indicator on the aptamers, which could affect the affinity of the aptamers and target molecules. Substantial research efforts are focused on the development of a 'label-free' aptasensor to overcome these shortcomings. Impedance spectroscopy is the most frequently used method in the development of a 'label-free' electrochemical aptasensor for monitoring the interaction between aptamers and biomolecules at the electrode surface, which possesses excellent advantages, such as high sensitivity, easy of use and using simple equipment.[24–27]

In this study, we developed a novel 'signal on' electrochemical impedance spectroscopy (EIS) biosensor for the label-free detection of 17β-estradiol. The principle of the electrochemical impedance biosensor was outlined in Fig. 1. The thiolated 17β-estradiol aptamer was self-assembled on the Au electrode surface through Au–S covalent bonds and formed a monolayer. In order to allow the aptamer probe to be well immobilized on the gold electrode surface,

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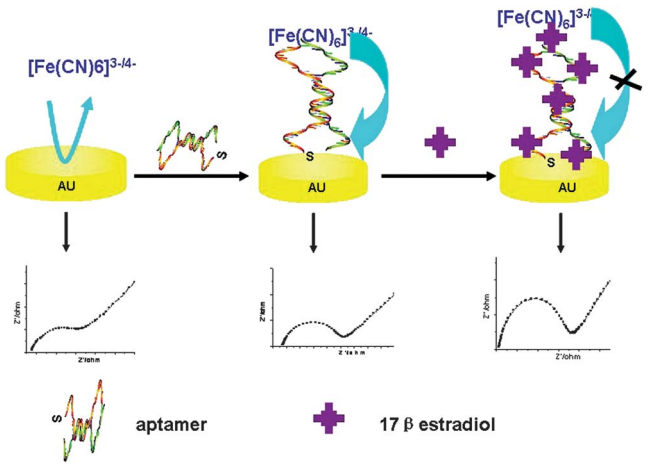

Fig. 1 Schematic illustration of label-free EIS biosensor for 17β-estradiol.

an alkanethiol moiety was modified at the 5′-end of the 76-mer DNA aptamer. The negatively-charged aptamer probe hindered the electron transfer reaction of the redox probe $Fe(CN)_6^{3-/4-}$ on the Au electrode surface. Then the aptamer-modified electrode was incubated with the target compound (17β-estradiol), and 17β-estradiol would bind with the aptamer on the electrode surface, then the electrode surface formed an analogous insulating layer which retarded the interfacial electron transfer reaction of the redox probe further, leading to the increase of the electron-transfer resistance. And the increase of electrochemical impedance can be represented by 17β-estradiol concentration indirectly; therefore, a sensitive biosensor for 17β-estradiol detection can be developed.

2. Experimental

2.1 Reagents

The single-strand DNA aptamer was chosen according to the prior reported literature;[11] then, it was synthesized by Sangon Inc. (Shanghai, China) and used as received; the sequence of the 76-mer-sized thio-labeled aptamer is given below:

5′-SHGCTTCCAGCTTATTGAATTACACGCAGAGGGTAGCGGCTCTGCGCATTCAATTGCTGCGCGCTGAAGCGCGGAAGC-3′

17β-Estradiol, sodium monohydrogen phosphate, sodium dihydrogen phosphate, potassium ferricyanide, potassium ferrocyanide, 2-methoxynaphthalene and 1-aminoanthraquinone were purchased from Sigma (St. Louis, MO); commercial milk samples were randomly collected from a supermarket. All chemicals were of analytical grade and used as received. Various concentrations of 17β-estradiol had been received by dissolving standard 17β-estradiol samples in 100 mmol L^{-1} tris HCl buffer (pH 8.0, containing 200 mmol L^{-1} NaCl, 25 mmol L^{-1} KCl, 10 mmol L^{-1} $MgCl_2$ and 5% ethanol). DNA buffer solution was prepared by dissolving DNA into 0.05 mol L^{-1} (pH 7.4) phosphate buffer solutions (PBS). Deionized water (18.4 MΩ) purified by a Milli-QTM system (Millipore) was used throughout the experiments. The urine samples were gotten from Fuzhou University Affiliated Hospital, Fuzhou, China. All experiments were performed in compliance with the relevant laws and institutional guidelines, and the institutional committee have approved the experiments.

2.2 Sensor preparation

The gold electrodes (3 mm in diameter) were pretreated according to the reported procedures.[28] Briefly, the gold electrodes were first polished with various particle size (0.05 and 1.0 μm) alumina slurry (Al_2O_3), followed by washing with Milli-Q water for 5 min and dried in a nitrogen stream to obtain clean gold electrode surfaces, then they were activated and further cleaned in a fresh 0.5 mol L^{-1} H_2SO_4 solution by performing the cyclic voltammetry (CV) method until ideal CV grams were obtained. Finally, the solution containing 10 μmol L^{-1} thiolated aptamer probe was dispensed on the electrode surface in a closed container for 2 h at 37 °C. The aptamer immobilized electrodes were incubated with different concentrations of 17β-estradiol at room temperature for 4 h for following assay.

2.3 Impedance detection

All electrochemical measurements were carried out by using a CHI Model 660D electrochemical analyzer (Chenhua Instruments, Shanghai, China), electrochemical impedance spectroscopy (EIS) studies were performed in 5 mmol L^{-1} $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ solution containing 0.1 mol L^{-1} KCl with a three-electrode system: a gold electrode used as the working electrode after being pre-immobilized with aptamer, an Ag/AgCl electrode and a Pt wire used as the reference electrode and counter electrode, respectively. Impedance measurements were recorded between 0.1 MHz and 0.1 Hz at a sinusoidal voltage perturbation of 5 mV amplitude. A Randles equivalent circuit was used to fit the obtained impedance spectra.

3. Results and discussion

3.1 Detection of 17β-estradiol

The incubation time has a significant effect on the detected impedance. Fig. 2 shows the electrochemical impedance of the proposed biosensors at different incubation times in 1.0×10^{-9} mol L^{-1}

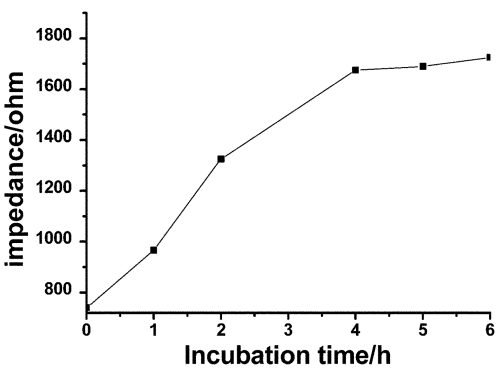

Fig. 2 Curve of impedance varying with incubation time. The Au electrode was incubated at room temperature. The impedance spectra were recorded in a 5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 mol L^{-1} KCl, using a frequency range of 0.1 MHz–0.1 Hz, a bias potential of +0.225 V and an AC amplitude of 5 mV.

17β-estradiol solution at 37 °C: the impedance increased rapidly in the first 4 h, and then achieved equilibrium at 4 h. Compared with other aptamer-based biosensors, such as the thrombin biosensor,[29] the incubation time was a little longer, where the reason perhaps lies in that the aptamer binds slowly with the 17β-estradiol. In this study, 4 h was chosen for the following experiments.

Different concentrations of 17β-estradiol also cause the change of electrochemical impedance spectra. As shown in Fig. 3, the impedance increases with the increase of 17β-estradiol concentration until it reaches a maximal value. Under the optimized conditions, the change of impedance (ΔI) has a linear relationship with the 17β-estradiol concentration in the range of 1.0×10^{-8} to 1.0×10^{-11} mol L^{-1}. The linear regression equation can be expressed as

$$\Delta I = 317.05 \log C + 3860.7 \quad R = 0.9921$$

where ΔI is the impedance, in ohms, C is the concentration of 17β-estradiol, and R is the correlation coefficient.

The detection limit was 2.0×10^{-12} mol L^{-1} (defined as S/N = 3), which is one order lower than the previously published electrochemical biosensor.[10] And furthermore, the biosensor is much easier to construct. Compared with the nanobiosenor[11] based on aptamer utilizing functional polymers, the proposed biosensor is easily achieved and displays a better sensitivity.

3.2 Selectivity and stability of the biosensor

In order to evaluate the specific response of the proposed biosensor to 17β-estradiol, two small organic chemicals (2-methoxynaphthalene and 1-aminoanthraquinone) which bear structural similarity to 17β-estradiol were chosen as control samples. Control experiments were performed by detecting the electrochemical impedance of the functionalized electrode in the presence of the two control samples. Their concentration was 5×10^{-11} mol L^{-1}. As shown in Fig. 4, only 17β-estradiol can cause an obvious change of impedance values. While the functionalized electrode was exposed to the solution containing two control samples, only a slight change in impedance signal was observed. These results confirmed that the proposed aptasensor has a good specificity for the target molecule detection. The reproducibility of the proposed aptasensor was also examined by detecting the impedance spectra of the different functionalized electrodes at the same concentration (5×10^{-11} mol L^{-1}) of 17β-estradiol. The relative standard deviation (RSD) of the resulting impedances did not exceed 5.0% ($N = 5$). This result indicates that the proposed aptasensor has good reproducibility.

The regeneration of the used biosensor was performed according to the literature.[30] The electrode surface had been rinsed with 50 mM EDTA to dissociate the aptamer–analyte complex, leading to 17β-estradiol being completely released from the aptamer-immobilized electrode surface. Then the biosensor recovered to its original state and could be ready for 17β-estradiol detection again, and the experimental results showed that the regenerated biosensor kept the same response as the original one and that it could be reused at least 10 times without obvious change. This clearly indicates that the regeneration of the biosensors is feasible.

3.3 Sample analysis

The developed biosensor has been applied to detect 17β-estradiol in urine samples. The urine samples were gotten from three volunteers and diluted 10^3 times by the buffer solution before determination without further pretreatment. The concentration of estradiol in the urine samples (average of three determinations) and the recovery of 17β-estradiol by the standard addition has been measured using the proposed biosensor; the results are shown in Table 1. The recoveries are between 91.7 and 103.1%, and the relative standard deviations are between 2.1 and 4.3% ($n = 3$). These results indicated that the proposed biosensor can be applied in real applications.

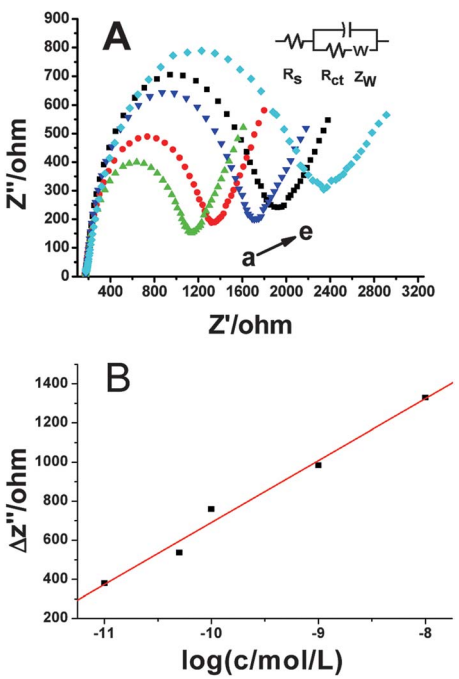

Fig. 3 (A) Impedance spectra (Nyquist plots) of electrodes incubated with different concentrations of 17β-estradiol: (a) 1×10^{-11} mol L^{-1}; (b) 5×10^{-11} mol L^{-1}; (c) 1×10^{-10} mol L^{-1}; (d) 5×10^{-9} mol L^{-1}; (e)1×10^{-8} mol L^{-1}. (B) Linear relationship between the change of impedance (ΔI) and concentration of 17β-estradiol. The conditions are the same as in Fig. 2.

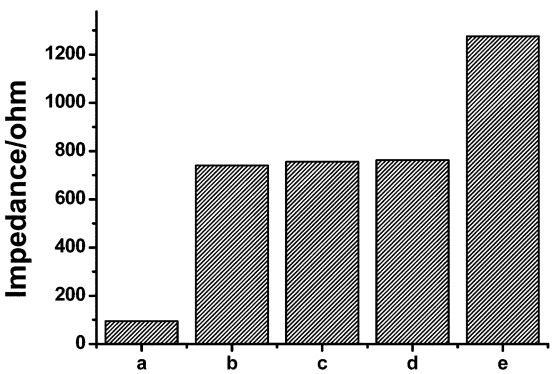

Fig. 4 Specificity of the EIS aptamer biosensor. Nyquist plots obtained under different conditions in control experiments: (a) bare Au electrode; (b) bare Au electrode modified with the DNA aptamer; (c) modified electrode incubated with 1-aminoanthraquinone; (d) modified electrode incubated with 2-methoxynaphthalene; (e) modified electrode incubated with 17β-estradiol. The conditions are the same as in Fig. 2.

Table 1 Determination of 17β-estradiol in urine samples[a]

Samples	Detected/mol L^{-1}	Added/mol L^{-1}	Found after adding/mol L^{-1}	Recovery (%)	RSD (%) ($n = 3$)
Volunteer 1	2.47×10^{-10}	1.20×10^{-10}	3.57×10^{-10}	91.7	3.8
Volunteer 2	2.26×10^{-10}	2.10×10^{-10}	4.35×10^{-10}	99.5	4.4
Volunteer 3	1.82×10^{-10}	1.59×10^{-10}	3.43×10^{-10}	101.3	3.2

[a] The sample from each volunteer had been detected separately.

4. Conclusions

In summary, a label-free electrochemical impedance spectroscopy biosensor for 17β-estradiol has been developed with high sensitivity and specificity. The recognition of small molecules by the aptamer-based electrode resulted in a larger electron transfer resistance for the $Fe(CN)_6^{3-/4-}$ probe by the EIS technique, and the difference of the signal before and after addition of targets shows a linear increase with the concentration of 17β-estradiol. Additionally, the biosensor has some distinct advantages, such as ease of handling, high stability and ease of re-use. Moreover, the biosensor provided satisfactory results for the detection of 17β-estradiol in urine samples.

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