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Aptamer-based electrochemical biosensor by using Au-Pt nanoparticles, carbon nanotubes and acriflavine platform

Zeinab Sahraei Beiranvand [a], Amir Reza Abbasi [a], Sara Dehdashtian [b], Ziba Karimi [a], Azadeh Azadbakht [a, *]

[a] Department of Chemistry, Khorramabad Branch, Islamic Azad University, Khorramabad, Iran
[b] Department of Chemistry, Islamic Azad University, Omidieh Branch, Omidieh, Iran

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ABSTRACT

Herein, an ultrasensitive electrochemical aptasensor for quantitative detection of bisphenol A (BPA) was fabricated based on a novel signal amplification strategy. This aptasensor was developed by electrodeposition of gold-platinum nanoparticles (Au-PtNPs) on glassy carbon (GC) electrode modified with acid-oxidized carbon nanotubes (CNTs-COOH). In this protocol, acriflavine (ACF) was covalently immobilized at the surface of glassy carbon electrode modified with Au-PtNPs/CNTs-COOH nanocomposite. Attachment of BPA-aptamer at the surface of modified electrode was performed through the formation of phosphoramidate bonds between the amino group of ACF and phosphate group of the aptamer at 5′end. By interaction of BPA with the aptamer, the conformational of aptamer was changed which lead to retarding the interfacial electron transfer of ACF as a probe. Sensitive quantitative detection of BPA was carried out by monitoring the decrease of differential pulse voltammetric (DPV) responses of ACF peak current with increasing the BPA concentration. The resultant aptasensor exhibited good specificity, stability and reproducibility, indicating that the present strategy was promising for broad potential application.

1. Introduction

Bisphenol A (BPA), as an important industrial chemical is widely used for the manufacture of polycarbonate plastics, epoxy resins, dental sealants, flame retardants, and food packaging. These kinds of materials are utilized as inner coating of food-storage or packaging materials, such as feeding bottles, food cans, water bottles, and tableware [1–3]. The global demand for BPA increased from 3.2 million tons in 2003 to 5.5 million tons in 2011 [4,5]. When these materials expose to heat, the unstable and lipophilic compound BPA can migrate into drinking water and food by leaching from food storage and packaging materials, or release into environment through wastewater discharged from plastic-producing industry; thus, humans may routinely ingest trace amounts of BPA [6]. Extensive studies have indicated that BPA is an endocrine-disrupting chemical, which interfere with thyroid function, central nervous system, endocrine pancreas, immune system, reproduction system and hormonal activities in the growth, development and reproductive processes of aquatic animals [7]. Numerous types of cancers, cardiovascular disease, and diabetes are associated with the intake of BPA [8]. Therefore, development of specific, reliable, sensitive, and accurate method for determination of trace amount of BPA has attracted particular attention for public health and environmental security.

Various conventional analytical technologies have been developed for BPA determination such as liquid chromatography-mass spectrometry [9], liquid chromatography-tandem mass spectrometry [10], gas chromatography-mass spectrometry [9,11], liquid chromatography-coulometric detection [12], liquid chromatography-fluorescence detection [13,14], and liquid chromatography-UV detection [15]. All these techniques are restricted with extensive extraction, costly instruments, complicated and time-consuming sample preparation, skillful and experienced technician, and cleanup step.

Also, enzyme-linked immunosorbent assay has been employed for BPA detection with the advantages of high sensitivity, favorable selectivity, and possible analysis of complex matrices without extensive pretreatment [16]. Immunoassay-based methods are

* Corresponding author.
E-mail address: Azadbakht.a@gmail.com (A. Azadbakht).

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strongly dependent on the quality of the prepared antibody, which has been challenging due to the instability of the antibody and nonspecific binding to BPA analogs [17]. Hence, exploring a more efficient and selective technique for BPA detection is definitely necessary; consequently, colorimetric, optical and electrochemical sensors have been designed for BPA detection by employing aptamers as the molecular recognition elements [18–23].

Aptamers are nucleic acid ligands that can specifically recognize their targets. They isolate from a synthetic nucleic acid pool by Systematic Evolution of Ligands by EXponential enrichment (SELEX) and can specifically bind to a variety of target molecules such as protein, small molecules, amino acids and even cells [24–29]. They are being an alternative to antibodies owning to the superior advantages over antibodies including temperature stability, easy artificial synthesis, specificity, low cost, and reusability. There is a growing interest for usage of aptamer in biorecognition area. Thus, fabrications of optic [30], fluorimetry [31], and electrochemical [32] aptasensors have received a significant attention.

How to convert the binding events between aptamer and target into a detectable electrochemical signal is the crucial point in the construction of electrochemical aptasensors. Generally, additional electroactive tags such as ferrocene derivatives, methylene blue, and ruthenium complexes [33–35], etc. have been employed to label aptamer to have the measurable signal. But labeling aptamer is a complicated and time-consuming process, even which might affect the affinity of aptamer toward target. Besides, electroactive compounds such as $[Fe(CN)_6]^{3-/4-}$ can also be directly added into electrochemical cell to produce a measurable signal. Adding the redox probe directly in the test solution is usually prone to false-positive results produced by the limitations, including initial sensing interface contamination and additional incubations in the $[Fe(CN)_6]^{3-/4-}$ between each measurements for a long time [36].

In the current paper, in order to overcome effectively these disadvantages of labeling aptamer or adding additional probe into test system, the acriflavine (ACF) as a redox probe was selected and in situ designed on the electrode surface due to its outstanding properties such as significant stability and good peak shape. CNTs-COOH decorated with gold–platinum nanoparticles (Au-PtNPs) was used as an effective immobilization matrix for covalent immobilization of ACF. Attachment of BPA-aptamer at the surface of modified electrode was performed through the formation of phosphoramidate bonds between the amino group of ACF and phosphate group of the aptamer at 5′end. Sensitive quantitative detection of BPA was carried out by monitoring the differences of the DPV response of the modified electrode before and after adding different concentration of BPA. The interaction of BPA with the aptamer caused the aptamer to be folded and the formation of BPA-aptamer complexes at the sensing interface hindered the interfacial electron transfer of the probe, resulting in the decrease of the electrochemical signal. Under the optimum conditions the prepared aptasensor had a wide linear response range, which proved that this method can be considered as an efficient method for constructing BPA electrochemical aptasensor with highly sensitivity and specificity.

2. Experimental

2.1. Chemicals

Multiwall carbon nanotubes with 95% purity, 10–20 nm diameters, and 1 μm length were obtained from Nanolab (Brighton, MA). Potassium ferricyanide ($K_3Fe(CN)_6$), potassium ferrocyanide ($K_4Fe(CN)_6$), hydrogen tetrachloroaurate ($HAuCl_4 \cdot 4H_2O$), hexachloroplatinate (IV) hydrate ($H_2PtCl_6 \cdot 5H_2O$), nitric acid, ethanol, dimethyl sulfoxide (DMSO), and potassium chloride were obtained from Merck (Germany) and Fluka. N-hydroxysuccinimide (NHS), acriflavine, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and bisphenol A were purchased from Sigma and used as received without further purification. The BPA-aptamer with the sequence of 5′-CCG GTG GGT GGT CAG GTG GGA TAG CGT TCC GCG TAT GGC CCA GCG CAT CAC GGG TTC GCA CCA-3′ was adopted according to the reported study [37]. All other chemicals were of analytical reagent grade and used without further purification. Phosphate buffered solution (PBS) was served as working buffer throughout the experiment, containing 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4 and 0.1 M KCl. All experiments were performed at ambient temperature of 25 ± 1 °C. Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and differential pulse voltammetry (DPV) were recorded to study the fabrication of modified electrode. The EIS measurement was performed in the presence of 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) and 0.1 M PBS (pH = 7.4). DPV was recorded with modulation amplitude of 0.05 V, a pulse width of 0.05 s, and sample width of 0.0167s.

2.2. Apparatus

All electrochemical measurements were performed via using a μAutolab III potentiostat/galvanostat (Eco Chemie, The Switzerland). The experimental conditions were controlled with NOVA 1.8 and Frequency Response Analyzer (FRA) software. A conventional three electrode cell was employed with an Ag/AgCl electrode (KCl 3 M) as the reference electrode, a Pt wire as counter electrode and a modified glassy carbon (GC) electrode as working electrode. The cell was a one compartment cell with an internal volume of 10 ml. The pH was measured with a JENWAY pH meter (model 3345). The size and morphology of materials were investigated by taking scanning electron microscopy (SEM), energy-dispersive X-ray (EDS) spectroscopy and wavelength-dispersive X-ray (WDX) (X-30 Philips, Philip's Company, Netherlands).

2.3. Electrode treatment and aptamer immobilization

Prior to the modification, the GC electrode was polished with 0.3 and 0.05 μm alumina slurry on a polishing cloth, followed by successive sonication in 1: 20 (v/v) nitric acid and ethanol to obtain a mirror-like surface. Then the GC electrode was cleaned in doubly distilled water in order to remove absorbed particles. After being dried in air, 10 μL of DMSO/CNTs-COOH solution (0.4 mg mL^{-1}) was cast at the surface of GC electrode to form a CNTs-COOH film at electrode surface. In order to prepare metal nanostructure film on the surface of electrode, electrodeposition is often used [38,39] as an easy and controllable method which allows electrodeposited film tightly attaches at the surface of electrode. Therefore, the CNTs-COOH/GC electrode was immersed in 0.2 M Na_2SO_4 solution containing 1 mM $HAuCl_4$ and 1 mM H_2PtCl_6 solution for potentiostatic electrodeposition at a potential of −0.2 V for 400 s. During this process, adsorbed $PtCl_6^{2-}$ and $AuCl_4^-$ at the surface of CNTs-COOH film were changed into Au-PtNPs (the electrode denoted Au-PtNPs/CNTs-COOH/GC) as displayed in Fig. 1. For comparison, AuNPs/CNTs-COOH/GC electrode was also constructed by immersing the CNTs-COOH/GC modified electrode in the solution containing 1 mM $HAuCl_4$ and 0.2 M Na_2SO_4 and the constant potential of −0.2 V for 400 s was applied. Afterward, the electrode was thoroughly rinsed with water and kept at room temperature for further use. For ACF immobilization, the Au-PtNPs/CNTs-COOH/GC modified electrode was immersed in the solution containing 10 mM EDC for 1 h. The EDC-attached electrode was washed and subsequently incubated in a 3.5 μM ACF solution for 2 h at room temperature. In this process the ACF was immobilized on the modified electrode through the covalent amide bonds formed by