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Extraordinary tunable dynamic range of electrochemical aptasensor for accurate detection of ochratoxin A in food samples

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Abstract

We report the design of a sensitive, electrochemical aptasensor for detection of ochratoxin A (OTA) with an extraordinary tunable dynamic sensing range. This electrochemical aptasensor is constructed based on the target induced aptamer-folding detection mechanism and the recognition between OTA and its aptamers results in the conformational change of the aptamer probe and thus signal changes for measurement. The dynamic sensing range of the electrochemical aptasensor is successfully tuned by introduction of free assistant aptamer probes in the sensing system. Our electrochemical aptasensor shows an extraordinary dynamic sensing range of 11-order magnitude of OTA concentration from 10^{-8} to 10^{2} ng/g. Of great significance, the signal response in all OTA concentration ranges is at the same current scale, demonstrating that our sensing protocol in this research could be applied for accurate detections of OTA in a broad range without using any complicated treatment of signal amplification. Finally, OTA spiked red wine and maize samples in different dynamic sensing ranges are determined with the electrochemical aptasensor under optimized sensing conditions. This tuning strategy of dynamic sensing range may offer a promising platform for electrochemical aptasensor optimizations in practical applications.

Keywords: Electrochemical aptasensor; Tunable dynamic detection range; OTA detection; Extraordinary dynamic range

1. Introduction

Presently, extensive studies have been carried out to improve the detection limit of existing sensing protocols in the field of analytical science [1–3]. For example, enzymatic catalysis reactions and in vitro nucleic acid amplification techniques have been widely integrated with conventional detection protocols to improve the sensitivity [4–7]. Meanwhile, due to the intrinsic advantages of nanomaterials, various functional nanoparticles have also been adopted for signal amplification [8–10]. As one of the most classic and efficient techniques, electrochemical biosensors have attracted extraordinary attention since the successful commercialization of the personal glucose meter [11–13]. Furthermore, with the occurrence of aptamers as the recognition probes, the electrochemical aptasensors have been further widely utilized for rapid and sensitive detections in many significant fields [7,14–16]. As mentioned, extensive researches have been executed to improve sensing performances especially detection limit of electrochemical biosensors [4,7,16–18]. However, from the perspective of practical applications, not only the detection limit, but also the dynamic sensing range of methods plays equally significant roles. And previous studies have clearly demonstrated that fixed dynamic range greatly restricts the performance of the electrochemical biosensors in many applications such as the monitoring of viral loading, in which the concentra-

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tion of the target could vary over many orders of magnitude [19].

It is important to note that for the current signal amplification protocols, there is a trade-off between the sensing range and the detection limit. People have to make wise choices of signal amplification protocols for achievement of satisfied sensing range or detection limit. Briefly, for signal-off model protocols, the sensing range would be extended toward the lower concentrations with signal amplification treatments [7,17]; while for signal-on model protocols, the sensing range would be extended toward both high and low concentrations due to the amplified sensing signals [18,20,21]. Because of this dilemma, the sensing signal could not be amplified unlimitedly even with the most extraordinary amplification treatments. In short, for electrochemical biosensors working in either sensing models, the sensing range can only be extended to a certain estimated extent based on the increased signal intensity.

Till now, only few research work on tunable dynamic range of electrochemical biosensors have been reported [3,22–24]. For example, Plaxco et al. first reported the expansion of detection range from the original 81-fold to more than 1000-fold by combining biosensors with identical specificity but differing affinities [22]. Following, Ricci et al. were able to tune the dynamic range of an E-DNA sensor by changing the stability of the stem-loop structured recognition element [19]. Besides, Lai et al. achieved the tunable dynamic range of an electrochemical biosensor for gold (III) detection by simply changing the length of the recognition DNA probe [24]. Interestingly, the dynamic range modulation in all reported research were successfully carried out with simple protocols. However, all the above reported research took advantage of the stability or rigidity of double stranded DNA and thus the target analytes are only limited to nucleic acids and metal ions. For detection of many important small molecules including toxins, antibiotics and medicine residues et al., related researches have rarely been reported. Actually, with the continuous screening and reporting of aptamers against common small molecules [25], similar research should be paid further attention due to the easy applications by replacing recognition nucleic acid probes with the aptamers of known sequences.

Herein, in this study, we report an innovative yet straightforward approach to tune the dynamic detection range of an electrochemical aptasensor. Firstly, we make an electrochemical aptasensor for detection of ochratoxin (OTA) as a model target, which has been extensively studied for the excellent recognition between OTA and its aptamer [26,27]. This kind of electrochemical aptasensors are mostly based on recognition induced folding of aptamer and subsequent signal variations [14]. Compared with traditional signal amplification based tune strategy, no amplification strategy is needed in our research in order to tune the dynamic detection range. Next based on the aptamer modified sensing interface of the electrode, we added assistant aptamer recognition probes into the sensing system to act as a tuning component for the tuning of detection range. Through this simple technique, the detection range was precisely controlled and modulated from 10^{-8} ng/g to 100 ng/g, over 13 orders of concentration magnitude. More importantly and of greater significance, in such a wide range (13 orders of concentration magnitude), all current response ratios at different analyte concentrations are almost at the same scale (defined as the valid signal variation zone), which means that this non-amplified sensing current (at the level of traditional biosensors) was also tuned for easy determination of target analyst at wide-range concentrations. To our best knowledge, this study is the first report about this kind of dynamic sensing range modulation in such a wide range without signal amplifications. And this dynamic sensing range modulation strategy could be easily transferred to detection for other analytes by changing the sequence of aptamer probes.

2. Experimental

2.1. Materials and methods

All the single-stranded oligonucleotide probe (ssDNA) and thiol-modified ssDNA were all synthesized by the Sangon Bioengineering (Shanghai) Co. Ltd. China. The detailed sequences of different oligonucleotide probes were as follows: free aptamer (probe 1), 5′-GAT CGG GTG TGG GCG TAA AGG GAG CAT CGG ACA-3′; immobilized aptamer (probe 2), 5′-thiol-(CH2)6-AAA GAT CGG GTG TGG GCG TAA AGG GAG CAT CGG ACA-Ferrocene-3′. 6-Mercapto-1-hexanol (MCH), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), and ochratoxin (OTA) were purchased from J&K Chemical, Shanghai. All other reagents were obtained from Sinopharm Chemical Reagent Company, China. Various buffer solutions were prepared from analytical grade chemicals without further purification and were prepared with ultrapure water (>18 MΩ, Milli-Q A10 system, Millipore, U.S.A). Storage buffer used for oligonucleotides was Tris-HCl (10 mM, pH 8.0) containing 1 mM ethylene diamine tetra-acetic acid (EDTA). Detection buffer was 100 mM pH 7.4 phosphate buffer containing 0.5 M sodium chloride.

2.2. Instrumentation

Cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and differential pulse voltammetry (DPV) measurements were carried out on a CHI 660D electrochemical workstation with a conventional three-electrode system composed of a functionalized gold electrode as the working electrode, a platinum wire auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode. EIS measurement was performed in 0.1 M KCl solution containing 5 mmol/L $[Fe(CN)6]^{3-/4-}$, and the frequency range was from 1 to 10^5 Hz at 0.2 V. DPV measurement was carried out in 10 mL detection buffer, and the experiment parameters were as follows: initial potential, 0.6 V; final potential, −0.1 V; pulse amplitude, 0.05 V; pulse interval, 0.05 s; sampling interval, 0.0167 s.

2.3. Fabrication of the sensing interface of OTA

We treated the electrode following the routine protocol of our group as described in [7,16]. In more details, before the modification of ssDNA probes, the electrode was immersed in