



A highly selective electrochemical impedance spectroscopy-based aptasensor for sensitive detection of acetamiprid



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ABSTRACT

A simple aptasensor for sensitive and selective detection of acetamiprid has been developed based on electrochemical impedance spectroscopy (EIS). To improve sensitivity of the aptasensor, gold nanoparticles (AuNPs) were electrodeposited on the bare gold electrode surface by cycle voltammetry (CV), which was employed as a platform for aptamer immobilization. With the addition of acetamiprid, the formation of acetamiprid–aptamer complex on the AuNPs-deposited electrode surface resulted in an increase of electron transfer resistance (R_{et}). The change of R_{et} strongly depends on acetamiprid concentration, which is applied for acetamiprid quantification. A wide linear range was obtained from 5 to 600 nM with a low detection limit of 1 nM. The control experiments performed by employing the pesticides that may coexist or have similar structure with acetamiprid demonstrate that the aptasensor has only specific recognition to acetamiprid, resulting in high selectivity of the aptasensor. The dissociation constant, K_d of 23.41 nM for acetamiprid–aptamer complex has been determined from the differential capacitance (C_d) by assuming a Langmuir isotherm, which indicates strong interaction between acetamiprid and aptamer, further proving high selectivity of the aptasensor. Besides, the applicability of the developed aptasensor has been successfully evaluated by determining acetamiprid in the real samples, wastewater and tomatoes.

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1. Introduction

Acetamiprid belongs to the new neonicotinoid class of systemic broad-spectrum insecticides. Due to the relatively low and chronic mammalian toxicity, and no long-term cumulative toxicity, it has been used to take the place of organophosphorus and other conventional insecticides for controlling the insects such as Hemiptera, Thysanoptera, and Lepidoptera on agricultural products (Seccia et al., 2005). However, considering it is widely used, there is still potential health risk to human beings who are exposed to the primary route of food and water polluted by acetamiprid. Therefore, it is highly desirable to develop a simple, fast, highly sensitive and selective method for quantification of acetamiprid residues in food and environment.

Current methods mainly include high-performance liquid chromatography (HPLC) (Liu et al., 2010; Seccia et al., 2008; Xie et al., 2011), gas chromatography (GC) (Zhang et al., 2008, 2010) and enzyme-linked immunosorbent assays (ELISAs) (Kim et al., 2004; Sugawara et al., 1998). However, both HPLC and GC involve expensive instruments, time-consuming sample preparation and

trained personnel. ELISAs have advantages of rapidness and simplicity, but some disadvantages cannot be neglected. For instance, ELISAs are susceptible to interferences from organic solvent or matrix components. Compared to these technologies, electrochemical method has been recognized as one of the most promising technologies because of its favorable portability, fast response, low cost, real-time detection and environmentally-friendly nature. However, acetamiprid is an electrochemically inert molecule. Currently, there is hardly any report about the utilization of electrochemical method for acetamiprid detection. As one of the electrochemical techniques, electrochemical impedance spectroscopy (EIS) is simple, sensitive and non-destructive, which has been widely used in development of biosensors (Rohrbach et al., 2012; Tran et al., 2011). Especially, EIS-based biosensors are well-suited to the detection of binding events happening on the transducer surface since minute changes in analytes to a biosensor surface can be easily and rapidly detected (Radi et al., 2005). However, high sensitivity of the method, being highly advantageous, can be also associated with nonspecific impedance changes that could be easily mistaken for specific interactions, that is, inability to discriminate between specific and nonspecific binding (Bogomolova et al., 2009). Therefore, it is very necessary to improve its selectivity by modifying electrode surface with particular substance that can specially interact with

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analytes. Thus, seeking for a recognition element, such as aptamer to bind acetamiprid specifically is the key for highly selective detection.

Aptamers are artificial functional oligonucleic acids selected *in vitro* through systematic evolution of ligands by exponential enrichment (SELEX) from random-sequence nucleic acids libraries (Ellington and Szostak, 1990; Tuerk and Gold, 1990). They are similar to antibodies, exhibiting high specificity and affinity for a wide range of target molecules such as protein (Fan et al., 2012; Hu et al., 2012; Li et al., 2011; Niu et al., 2012) small molecules (Ho et al., 2012), amino acids (Liang et al., 2011; Zhao et al., 2012), and even cells (Wang et al., 2010a; Zhou and Wang, 2012). Meanwhile, aptamers possess a variety of merits over antibodies, including smaller size, easier artificial synthesis and modification, and better stability. Thus, the combination of EIS and acetamiprid aptamer not only has excellent properties of electrochemical technique but also achieves high selectivity for acetamiprid detection. At the same time, EIS-based aptasensors are label-free, overcoming complex and laborious process of labeling aptamers which might affect the bioaffinity between aptamers and their targets. Therefore, EIS-based aptasensor is considered as an ideal choice for acetamiprid detection.

In addition, how to increase the amount of aptamer molecules anchored on electrode surface to obtain high sensitivity is a key problem in the process of aptasensor construction. An effective method is immobilizing metal nanoparticles on substrate material (Bamrungsap et al., 2012; Chen et al., 2010). Especially, gold nanoparticles (AuNPs) show some fascinating properties, such as favorable microenvironment, good biocompatibility and high electron transfer ability. They have been widely used as the immobilization matrix in biosensors fabrication for enhancing biosensor performance (Huo et al., 2011; Wang et al., 2010b). In this work, we combine aptamer with the recognition properties and EIS technique to develop a label-free acetamiprid aptasensor by immobilizing thiol-terminated aptamer on the AuNPs-deposited electrode surface. Acetamiprid captured by the aptamer probe is quantified via monitoring the changes of the electron transfer resistance (R_{et}) by EIS. The sensitivity and selectivity of the aptasensor developed is investigated in detail. The mechanism of selectivity is further discussed by calculating the dissociation constant for acetamiprid–aptamer complex formed on the AuNPs modified surface theoretically. Meanwhile, the stability and applicability of the aptasensor in real samples are also investigated.

2. Experiment

2.1. Reagents

Aptamer was chosen according to the prior reported literature (He et al., 2011). The oligonucleotides used were purchased from Genscript Biotechnology Co. (Nanjing, China) with the following sequences:

DNA sequence:

5'-(SH)-(CH₂)₆-TGTAATTTGTCTGCAGCGTTCTTGATCGCTGA-CACCATATTATGAAGA-3'

Mutated DNA sequence:

5'-(SH)-(CH₂)₆-TGTAATTTGTCTGCAGCGTTCTTGATCGCTGC-CACCTTAGTCTGCAGA-3'

Acetamiprid (99%) was purchased from Aladdin Chemistry Co., Ltd. 6-Mercap-1-hexanol (MCH) and 1,4-dithiothreitol (DTT) were obtained from Adamas Reagent Co. Ltd. Hydrogen tetrachloroaurate (III) hydrate (HAuCl₄) was purchased from Sigma-Aldrich. Herein, DTT was a reducing agent, which was used to break disulfide bond and ensure free -SH groups were ready to react

with the gold surface. Aptamer was dissolved in Tris–EDTA buffer (TE, pH 8.0) and kept frozen. 0.1 M phosphate buffer (PBS, pH 7.0) containing 5 mM K₃[Fe(CN)₆]/K₄[Fe(CN)₆] (1:1, v/v) and 0.1 M KNO₃ was chosen as supporting electrolyte for monitoring the different stages of the aptasensor fabrication and acetamiprid assays. Tris–HCl buffer (pH 7.41, containing 20 mM Tris–HCl/0.1 M NaCl/0.2 M KCl/5 mM MgCl₂) was prepared and stored at 4 °C. All chemicals are of analytical reagent grade.

2.2. Apparatus

CHI660C electrochemical workstation (Chenhua Instruments Co., Shanghai) was used for electrochemical measurements. A conventional three-electrode system was employed with aptamer modified gold electrode or bare gold electrode (diameter, 2 mm) as working electrode, saturated calomel electrode (SCE) as reference electrode, and platinum wire as counter electrode. The morphology of AuNPs/gold electrode and bare gold electrode was observed using scanning electron microscopy (FE-SEM, S-4800, Hitachi).

2.3. Design and fabrication of the aptasensor

According to the prior reported literature (Chen et al., 2011), the bare gold electrode was polished with 0.3 and 0.05 μm alumina powders. The polished electrode was put into a freshly prepared piranha solution (v:v 3:1 H₂SO₄/H₂O₂) for 5 min at 70 °C (Caution!), followed by ultrasonically cleaned with ethanol and double-distilled water, respectively. The electrode was electrochemically pretreated in 0.5 M H₂SO₄ by potential scanning between –0.2 and 1.5 V until a reproducible cyclic voltammogram was obtained. The fresh electrode was immersed into 2 mM HAuCl₄ containing 0.1 M KCl, where AuNPs were electrodeposited on the bare electrode surface (AuNPs/gold electrode) between –0.2 and –1.2 V, for 20 cycles by CV. Next, the AuNPs/gold electrode was incubated with Tris–HCl buffer (pH 7.41) containing 2 μM aptamer at 4 °C overnight, and it was rinsed absolutely with Tris–HCl buffer to remove physically absorbed aptamer. After the incubation, the fabricated aptamer/AuNPs/gold electrode was treated with 1 mM MCH for 15 min to block nonspecific sites, thoroughly rinsed and dried by N₂. Thus, any binding except the recognition binding between aptamer and acetamiprid was inhibited. The aptamer probe (MCH/aptamer/AuNPs/gold electrode) was obtained. Besides, a mutated aptamer based biosensor was fabricated with the same method except replacing the aptamer with a mutated sequence. Scheme 1 illustrates the schematic representation of the aptasensor construction and performance.

2.4. Experimental measurements

All electrochemical experiments were performed in an electrochemical cell containing 10 mL 0.1 M PBS (pH 7.0), 5 mM [Fe(CN)₆]^{3–/4–} and 0.1 M KNO₃. EIS measurements were recorded between 1000 Hz and 0.1 Hz with a sinusoidal voltage perturbation of 5 mV amplitude. The aptamer probe was soaked in a 20 μL-droplet of various concentrations of acetamiprid for 40 min at 37 °C, followed by thoroughly rinsing with Tris–HCl buffer to remove unbound acetamiprid. Before and after incubation in acetamiprid solution, the changes in impedance were recorded.

In the control experiments, the method was the same as that of acetamiprid detection, but acetamiprid was replaced with the same concentration of imidacloprid, 2,4-dichlorophenoxy acetic acid (2,4-D), atrazine, omethoate, chlorpyrifos and dipterex. The real samples, wastewater and tomatoes were analyzed by the aptasensor. Wastewater sample was collected from a sewage

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