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Acetamiprid multidetection by disposable electrochemical DNA aptasensor

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ABSTRACT

In this work, we propose an electrochemical DNA aptasensor for sensitive multidetection of acetamiprid based on a competitive format and disposable screen-printed arrays. To improve the sensitivity of the aptasensor, polyaniline film and gold nanoparticles were progressively electrodeposited on the graphite screen-printed electrode surface by cyclic voltammetry. Gold nanoparticles were then employed as platform for thiol-tethered DNA aptamer immobilization. Different acetamiprid solutions containing a fixed amount of biotinylated complementary oligonucleotide sequence by DNA aptasensor arrays were analyzed. Streptavidin-alkaline phosphatase conjugate was then added to trace the affinity reaction. The enzyme catalyzed the hydrolysis of 1-naphthyl phosphate to 1-naphthol. The enzymatic product was detected by differential pulse voltammetry. A decrease of the signal was obtained when the pesticide concentration was increased, making the sensor work as signal off sensor. Under optimized conditions by testing key experimental parameters, a dose-response curve was constructed between 0.25 and 2.0 μM acetamiprid concentration range and a limit of detection of 0.086 μM was calculated. The selectivity of the aptasensor was also confirmed by the analysis of atrazine pesticide. Finally, preliminary experiments in fruit juice samples spiked with acetamiprid were also performed.

1. Introduction

Acetamiprid is a neuro-active widespread pesticide of the neonicotinoid class, synthetic derivatives of nicotine. Neonicotinoids were introduced for agricultural control of sucking-type insects on leafy vegetables, fruits and tea trees in the early 90s and recently have become widely diffused representing around 24% of global market pesticide in 2008 [1]. Acetamiprid has shown a high toxicity, causing potential risk to humans who are exposed to the contaminated environment [2]. It has been classified as a probably non-carcinogenic substance but it is known also for its toxic effects, even chronic, for mammalians [2–4]. It acts as nicotinic acetylcholine receptor agonist, causing to the contaminated organism paralysis and then death [5]. Due to the mentioned frequent and extensive usage, the development of an efficient and reliable analytical method for its detection in food samples remains an important target in order to prevent potential risk for human health. The maximum residue limits (MRLs) in the European Union admitted in fruits and vegetables are periodically updated by the European Food Safety Authority (EFSA) [4,6]. Detection of neonicotinoid pesticides is usually carried out via conventional instrumental techniques based on chromatographic methods, like high-pressure liquid chromatography or gas chromatography, often coupled to mass spectrometry [7–9]. These techniques are time consuming and expensive; in addition, the instruments require highly skilled technicians for their use and maintenance. In recent years, biosensor development for pesticide analysis has grown intensively, proposing itself as a cheaper and easily alternative, especially for screening analysis [10,11]. To improve specificity and chemical stability of affinity biosensors, synthetic receptors such as molecularly imprinted polymers [12,13] and aptamers were successfully used [14,15]. Different electrochemical aptasensors for acetamiprid detection based on electrochemical impedance spectroscopy (EIS) were reported in literature [16,17]. In this work, we realized an electrochemical DNA aptasensor for acetamiprid detection based on a competitive assay format. The method combines a special chip containing eight screen-printed electrochemical cells with a portable, computer-controlled instrument. The novelty of the proposed aptasensor compared to those reported in literature was the use of aptamer coupled with nanocomposite polymeric film modified electrochemical platforms as a first step for sensitive, disposable and cost-effective screening multianalysis on field.

The aptamers used in this work were selected from a library of aptamers, designed by SELEX [18–20] that showed to have the ability to recognize acetamiprid [21,22]. The use of screen-printed

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cells is well known approach for the development of efficient biosensors, due to their relatively low cost, simplicity and rapidity of use coupled to small dimensions [23–27]. To improve sensitivity of the aptasensor, polyaniline film and gold nanoparticles were progressively electrodeposited on the graphite screen-printed electrode surface by cyclic voltammetry. Gold-polyaniline nanocomposite screen-printed electrodes were then modified with a mixed monolayer of a thiolated DNA aptamer and a spacer thiol, 6-mercapto-1-hexanol. Different acetamiprid solutions containing a fixed amount of biotinylated complementary oligonucleotide sequence with DNA aptasensor arrays were analyzed. An enzyme-amplified detection scheme, based on the coupling of biotinylated oligonucleotide and streptavidin-alkaline phosphatase conjugate was then applied. The enzyme catalyzed the hydrolysis of 1-naphthyl-phosphate to 1-naphthol. The enzymatic product is electroactive and was detected by Differential Pulse Voltammetry (DPV). This electrochemical technique was chosen because it offers a lower detection limit than linear sweep voltammetry, by increasing the ratio between faradic and non-faradic currents, sampling the current with a proper delay after the selected potential pulse [28]. A decrease of the signal was obtained when the pesticide concentration was increased, making the sensor work as signal off sensor.

Each step of the presented aptasensor was optimized and its analytical performances were studied. Finally, preliminary experiments in fruit juice samples spiked with acetamiprid were also performed.

2. Materials and methods

2.1. Chemicals

Perchloric acid ($HClO_4$), sulfuric acid (H_2SO_4), aniline, potassium ferrocyanide [$K_4Fe(CN)_6$], potassium ferricyanide [$K_3Fe(CN)_6$], streptavidin-alkaline phosphatase enzyme, di-sodium hydrogen phosphate (Na_2HPO_4), sodium di-hydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$) and formic acid have been purchased from Merck (Milan, Italy). Tetrachloroauric acid ($HAuCl_4$), 6-mercapto-1-hexanol (MCH), KCl, NaCl, $MgCl_2$, Bovine Serum Albumin (BSA), diethanolamine, Trizma® hydrochloride, DL-dithiothreitol (DTT), acetamiprid, acetonitrile and HPLC ultrapure water have been purchased from Sigma-Aldrich (Milan, Italy). All reagents were purchased at analytical grade. Milli-Q water were used throughout the work.

The DNA sequences, listed below, were purchased from Eurofins MWG Operon, Ebersberg, Germany:

thiol-tethered DNA aptamer (oligo1):

5′-(SH)-$(CH2)_6$-TGTAATTTGTCTGCAGCGGTTCTTGATCGCTGA-CACCATATTATGAAGA-3′ biotinylated oligonucleotide complementary sequence (oligo2):

5′-(biot)-TEG(triethylene glycol)-TCTTCATAATATGGTGTCAG-3′.

biotinylated oligonucleotide non-complementary sequence (oligo3):

5′-(biot)-TEG(triethylene glycol)- AGCTA-CATTGTCTGCTGGGTTTC-3′.

The buffer solutions used in this work are:

buffer A: 20 mM TRIS buffer pH 7.4, 0.1 M NaCl, 0.1 M KCl, 5 mM $MgCl_2$.

buffer B: 10 mM TRIS buffer pH 8.3.

buffer C: 0.1 M diethanolamine buffer (DEA) pH 9.6.

buffer D: 0.1 M phosphate buffer pH 7.0.

Thiol-modified probe was treated with dithiothreitol (DTT), purified by elution through NAP-5 columns (GE Healthcare life Sciences illustra NAP-5) and then quantified by measuring UV absorption at 260 nm. All oligonucleotide stock solutions have been prepared in TRIS buffer B and stored frozen.

2.2. Instrumentation

UV absorption measurements were carried out using a Varian Cary 100 Bio UV-spectrophotometer collecting the data with the related Cary WinUV thermal application 3.00(182) software.

Secondary structures of oligo1 and oligo2 sequences were predicted using the software Mfold [29–31].

Differential pulse and cyclic voltammetry measurements were carried out using the portable potentiostat/galvanostat PalmSens electrochemical analyzer (PalmSens, the Netherlands), and the results analyzed by PSTrace 2.3 software.

Electrochemical Impedance Spectroscopy (EIS) measurements were performed in a digital potentiostat/galvanostat AUTOLAB PGSTAT 30(2)/FRA2 controlled with General Purpose Electrochemical System (GPES) and Frequency Response Analyzer (FRA2) 4.9 software (Eco Chemie, Utrecht, the Netherlands).

The transducers used for the aptasensor development were screen-printed 8-sensor arrays, based on eight graphite working electrodes (diameter=2.0 mm), each one with its own silver pseudo-reference electrode and graphite counter electrode. The arrays were screen-printed in-house using a DEK 248 screen-printing machine (DEK, Weymuth, UK). A polyester film were used as supporting material (Autostat CT5) from Autotype (Milan, Italy). Polymeric inks Electrodag PF-410 (silver) and Electrodag 423 SS (graphite) were purchased from Acheson (Milan, Italy). Vinylfast 36–100, obtained from Agron (Lodi, Italy) was used as insulating ink.

HPLC measurement were carried out using a Waters Alliance 2690 separations module (Waters corporation, Milford USA), equipped with a Supelco Supelcosil™ LC-PAH (25 cm × 4.6 mm, i.d. 5 μm) column and coupled with a Waters DAD 996 detector (Waters corporation, Milford USA).

2.3. DNA melting curve studies

To evaluate the hybridization reaction between the DNA sequence (oligo1), used as capture aptamer, and the selected complementary sequence (oligo2), melting curves were recorded using the Varian Cary 100 Bio UV-spectrophotometer equipped with a 6+6 peltier thermostatable multicell holder and built-in temperature probes.

100 μL of 1.0 μM oligonucleotides solutions in TRIS buffer A were placed into quartz microcuvettes (10 mm path length). Measurements were carried out by increasing the temperature at a constant rate of 1.0 °C/min from 25 to 95 °C, monitoring the absorbance at 260 nm (spectral bandwidth: 1 nm). Temperature values were directly measured inside the cuvettes, using a probe immersed into the sample solutions. The instrumental accuracy of such measurements was within ± 0.05 °C. A TRIS buffer A solution was used as blank solution. T_m values were either acquired on independently prepared samples ($n \geq 4$) or by repetitive collection (through denaturation-reannealing cycles) on single samples ($n \geq 4$).

Melting temperatures were obtained as first-order derivate plot of Abs versus T using the Cary Thermal Analysis software.

2.4. Electrochemical measurements

Electrochemical impedance spectroscopy (EIS) measurements were performed in presence of 5.0 mM $[Fe(CN)_6]^{3-/4-}$ redox probe (equimolar solution in 0.10 M KCl and phosphate buffer D). A voltage of 10 mV in amplitude (peak-to-peak), within the frequency range 100 kHz–10 mHz, was superimposed to the applied bias potential. The dc potential was set up to +130 mV, the formal