Electrochemical DNA-Based Sensor for Organophosphorus Pesticides Detection

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Abstract In this work, we propose an electrochemical DNA-based sensor for sensitive detection of organophosphorus pesticides. To improve the sensitivity of the DNA-based sensor, 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 the immobilization of thiol-tethered DNA oligonucleotide sequence complementary to the selected biotinylated DNA aptamer for profenofos detection. Streptavidin-alkaline phosphatase enzyme conjugate was then added to trace the affinity reaction through the hydrolysis of 1-naphthyl phosphate to 1-naphthol, which was then 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.

Keywords Pesticide · Aptamer · DNA · Competitive assay · Biosensor

1 Introduction

Organophosphorus pesticides (e.g. phorate, profenofos, isocarbophos) are highly toxic substances that nowadays are still used outside EU in some harvesting protocols. This class of compounds, like some nerve agents, acts on the enzyme acetylcholinesterase as neuromuscular inhibitors, affecting normal functions in insects, but also in humans and many other animals [1].

Conventional analytical methods for organophosphorus pesticides are based on chromatographic techniques, which provide sensitive and selective detection. Despite these advantages, chromatographic techniques require highly skilled technicians for

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operation and they are not suitable for screening analysis. Therefore, there are continuing developments in rapid and cost-effective devices for environmental monitoring including in situ analysis [2, 3]. In this perspective, biosensor development for pesticide analysis becomes urgent and proposes itself as an easily alternative to conventional techniques for screening analysis [4]. The main difficulties about the use of biosensors for pesticides detection concern the possibility to obtain antibodies for targets with high toxicity. In this way, the use of synthetic receptors such as DNA aptamer has recently become an interesting alternative to the antibodies in affinity biosensors technology [5, 6].

In this work, we report preliminary experiments using an electrochemical DNA aptasensor for profenofos organophosphorus pesticide detection based on a competitive assay format. The method combines the portability of graphite screen-printed electrochemical cells and of a computer-controlled instrument. Herein, the proposed aptasensor exploits the use of oligonucleotides coupled with a built-in electrochemical platform consisting of a metallic nanoparticles/polymeric film nanocomposite for disposable and cost-effective screening in situ analysis.

The DNA aptamer used in this work was selected from a library of aptamers, designed by SELEX, that proved itself to show the highest ability to recognize profenofos [7].

2 Materials and Methods

Perchloric acid ($HClO_4$), sulfuric acid (H_2SO_4), aniline, streptavidin-alkaline phosphatase enzyme, di-sodium hydrogen phosphate (Na_2HPO_4), sodium di-hydrogen phosphate di-hydrate ($NaH_2PO_4{\cdot}2H_2O$), tetrachloroauric acid ($HAuCl_4$), 6-mercapto-1-hexanol (MCH), KCl, $MgCl_2$, BSA, di-ethanolamine (DEA) and profenofos have been purchased from Sigma-Aldrich (Italy). The DNA sequences (oligo-SH: 5′-(SH)-$(CH_2)_6$-CCGATCAAGAATCGCTGCAG-3′; apt-BIO: 5′-(biotin)-TEG(trietylene glycol)-AAGCTTGCTTTATAGCCTGCAGCGATTCTTGATCGGAAAAGGCTG AGAGCTACGC-3′) were purchased from Eurofins Genomics (Germany). Immobilization buffer: 0.5 M phosphate buffer, pH 7. Detection buffer: 0.1 M DEA buffer, 0.1 M KCl, 1 mM $MgCl_2$, pH 9.6. Milli-Q water was used for all preparations. Electrochemical measurements were carried out with a portable potentiostat/galvanostat PalmSens electrochemical analyser (PalmSens, the Netherlands), and the results analysed by PSTrace 2.3 software.

The graphite screen-printed electrodes (GSPEs) were modified with polyaniline (PANI) [8] and gold nanoparticles (AuNPs). Sensors were then modified by self-assembly of a mixed monolayer of thiolated DNA capture probe (oligo-SH) and MCH [9]. Then, a solution containing a proper concentration of biotinylated DNA aptamer (apt-BIO) and the target pesticide was dropped on the sensor surface and the competitive reaction was allowed to proceed. The biotinylated hybrids formed onto the developed aptasensor surface were coupled with a streptavidin-alkaline phosphatase conjugate and the enzymatic product thus formed (1-naphthol) was detected by differential pulse voltammetry (DPV).

3 Results

The primary surface modification of the graphite screen-printed working electrodes was obtained by electrodepositing via CV a polyaniline (PANI) layer, followed by gold nanoparticles electrodeposition (AuNPs) carried out via CV too, in accordance with previously reported studies. Cyclic voltammetry provides detailed information on electrode surface changes. Thus, it was used to characterize the layer-by-layer formation of PANI during the electropolymerization process (Fig. 1a, b). Cathodic and anodic peak current height was recorded at different number of cyclic scans. After

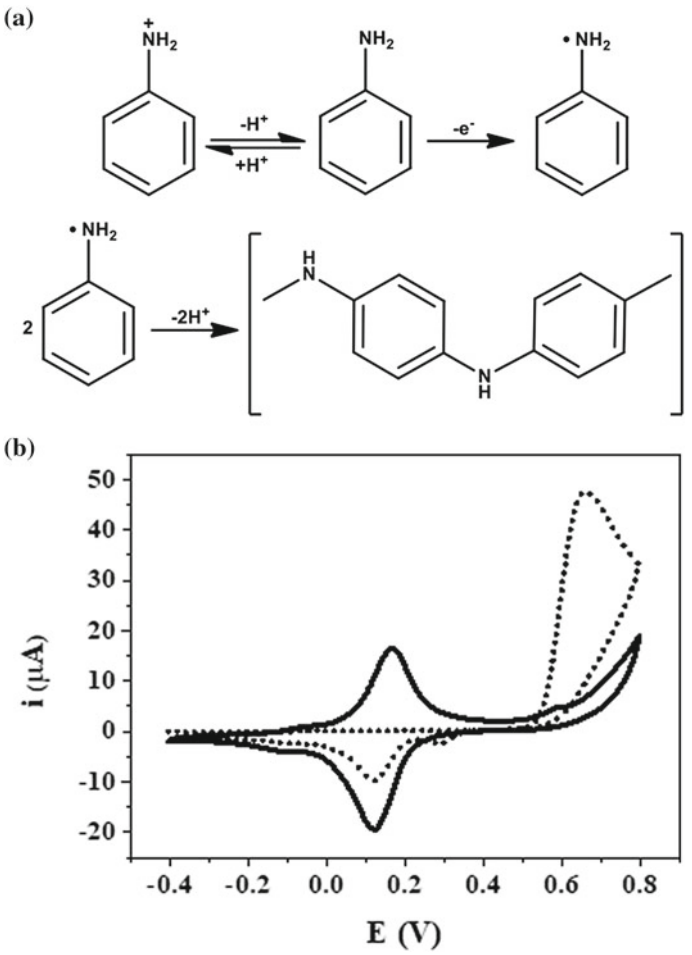

Fig. 1 **a** Aniline polymerization mechanism. **b** Aniline electropolymerization (first and last scans shown)

the 10th cycle, no further increase of peak current height was obtained; consequently, ten cyclic scans were then selected for further experiments.

Then, gold nanoparticles were electrodeposited on polyaniline modified graphite screen-printed electrodes (PANI/GSPEs) using CV in accordance with the optimized procedure previously reported (Fig. 2).

After the electrodeposition of PANI and AuNPs, the electrode conductivity increases of around 40% in comparison to bare GSPE surface.

Preliminary experiments were performed for profenofos pesticide (Fig. 3) detection by competitive assay. By analyzing a 5 μM profenofos solution, a decrease of 50% in the current peak height was obtained with respect to the blank value.

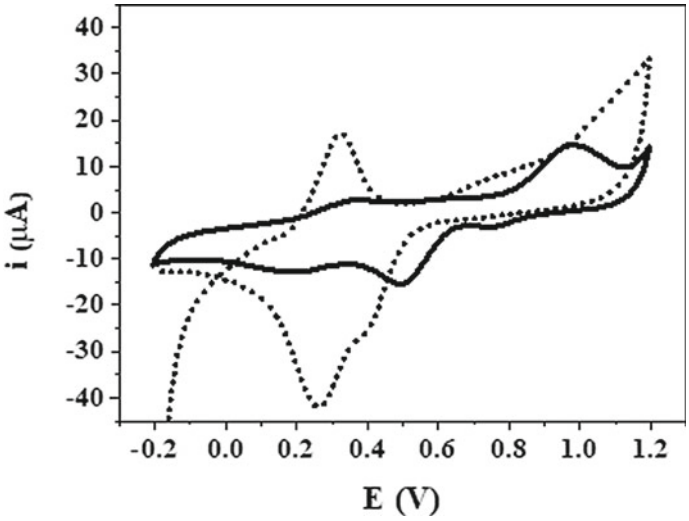

Fig. 2 Gold nanoparticles electrodeposition (first and last scans shown)

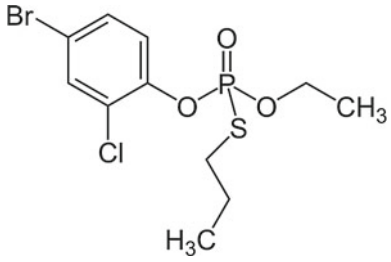

Fig. 3 Profenofos pesticide structure

4 Conclusions

The realized platform, based on graphite screen-printed electrodes (GSPEs) modified with polyaniline (PANI), gold nanoparticles (AuNPs) and an aptamer can contribute to profenofos detection as a valid and innovative analytical approach. The developed aptasensor allows the direct determination of profenofos, thanks to an easy to achieve, rapid and quite low-cost method.

These preliminary results encourage the application in the immediate future of this platform for pesticide detection, testing the developed assay for multiscreening analysis.

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