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Ultrasensitive aptamer biosensor for malathion detection based on cationic polymer and gold nanoparticles

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ABSTRACT

In this work, we have demonstrated a novel sensing strategy for an organophosphorus pesticide namely, malathion, employing unmodified gold nanoparticles, aptamer and a positively charged, water-soluble polyelectrolyte Polydiallyldimethylammonium chloride (PDDA). The PDDA when associated with the aptamer prevents the aggregation of the gold-nanoparticles while no such inhibition is observed when the aptamer specific pesticide is added to the solution, thereby changing the color of the solution from red to blue. This type of biosensor is quite simple and straightforward and can be completed in a few minutes without the need of any expensive equipment or trained personnel. The proposed method was linear in the concentration range of 0.5–1000 pM with 0.06 pM as the limit of detection. Moreover, the proposed assay selectively recognized malathion in the presence of other interfering substances and thus, can be applied to real samples for the rapid screening of malathion.

1. Introduction

Malathion (Diethyl 2-[(dimethoxyphosphorothioyl)sulfanyl] butanedioate), is a broad-spectrum insecticide used to control a variety of outdoor insects in both agricultural and residential settings. Malathion is registered for use on food, feed, and ornamental crops and in mosquito, boll weevil and fruit fly eradication programs. The widespread application of this pesticide has resulted in the serious contamination of drinking water due to its presence in soil and water at concentrations far exceeding its permissible limits, causing major health based concerns (Mionettol et al., 1994; Bouchard et al., 2010). Therefore, in order to control such a hazardous pesticide, a rapid yet sensitive method of detection is required urgently. For the detection of trace levels of pesticides, various analytical methods such as high-performance liquid chromatography, gas chromatography, thin-layer chromatography and gas chromatography-mass spectrometry are already being used (Brito et al., 2002; Berijani et al., 2006; Boyd-Boland et al., 1996; Liu et al., 2011). However, due to various problems associated with these methods such as complexity, long hours, cost and instrumentation, researchers are now shifting to low cost and rapid sensing strategies (Weerathunge et al., 2014). The last decade saw the rise of immunoassay based detection techniques as an alternative for the detection of pesticides at high sensitivity levels (Qian et al., 2009; Gabaldon et al., 2007; Jiang et al., 2011; Suri et al., 2009). However, the use of antibodies in such immunoassays still remains the major hurdle. This inadequacy of the above mentioned methods highlights the need for new, high-speed and sensitive techniques for the detection of pesticides. Aptamers have received huge attention in the past few years due to their applicability in various range of analytical applications. Aptamers are single stranded nucleic acid or peptide molecules of size less than 25 kDa which can be natural or synthetic by origin (Lau and Li, 2011; Lau et al., 2011). Aptamers are highly specific and selective towards their target compounds, namely, ions, proteins, toxins, microbes and viruses, due to their precise and defined three-dimensional structures (Wei et al., 2007; Kuang et al., 2011; Chen et al., 2013; Wu et al., 2012). In comparison with antibodies, aptamers based biosensors have many advantages such as – no use of animals, better stability, and enhanced specificity in various kinds of assays such as electrochemical (Liu et al., 2012; Ho et al., 2012), fluorescence (Zhang et al., 2014), chemiluminescence (Freeman et al., 2011), or colorimetric (Chen et al., 2013; Shi et al., 2013). Aptamers possess additional advantages such as conformational change on analyte-binding and high specificity for single target analyte. In conjunction with gold nanoparticles, aptamers serve as promising molecules for application as sensitive

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and specific bioprobes for the detection of pesticides. In such type of assays, AuNPs serve as colorimetric labels due to their Surface plasmon resonance (SPR) phenomenon (Lin et al., 2006). Although there have been reports regarding the detection of Arsenic (Wu et al., 2012) and thrombin protein (Chen et al., 2014) using AuNPs and polymer but, until now, colorimetric aptasensor for the detection of malathion using electrolyte has never been reported. Keeping these points in mind, in this paper, a sensitive and selective aptamer biosensor using electrolyte for the detection of malathion is presented. The aptamer sequence was derived from the literature where the detection of malathion was carried out using SERS (Barahona et al., 2013). On addition of positively charged electrolyte, the aptamer binds to it owing to its negatively charged backbone. However, when the pesticide, specific to the aptamer is present, the aptamer binds to the pesticide, and the electrolyte remains free to lead to the aggregation of the gold nanoparticles, imparting a color change from red to purple-blue.

2. Experimental

2.1. Materials and instrumentation

Hydrogen tetrachloroaurate (III) trihydrate, trisodium citrate dihydrate, Polydiallyldimethylammonium chloride (PDDA), aptamer and malathion were obtained from Sigma Aldrich (India). The oligonucleotide having the sequence 5′ATCCGTCACACCTGCTCTTATACACAATTGTTTTTCTCTTAACTTCTTGACTGCTGGTGTTGGCTCCCGTAT-3′ was desalted and HPLC purified. All the reagents used were of analytical grade and the experiments were performed in Milli-Q water having a resistivity of 18.2 MΩ cm. The glassware was rinsed with aqua regia prior to use.

2.2. Instrumentation

The UV-Visible measurements were recorded on JASCO V-530 spectrophotometer. TEM analyses were carried out using Hitachi H-7500 microscope. HPLC chromatograms were obtained from Waters HPLC using Waters 2996 Photodiode Array Detector.

2.3. Gold Nanoparticles Synthesis

AuNPs were synthesized by citrate reduction of chloroauric acid (Bala et al., 2015). Briefly, 100 mL of aqueous 0.01% $HAuCl_4$ solution was heated to boiling followed by the rapid addition of 2 mL of 1% trisodium citrate solution. The color change from pale yellow to red indicated the formation of AuNPs. The solution was further boiled for an additional 10 min and allowed to cool at room temperature under stirring. The colloids were stored in dark bottles at 4 °C.

2.4. Analysis of malathion

Stock solution of malathion was prepared in acetone and stored at 6° C. Varied dilutions of malathion were then prepared in phosphate buffer (pH 7.32). For malathion analysis, 10 µL of 50 nM aptamer was mixed with 50 µL of different malathion concentrations, diluted with 100 µL phosphate buffer and incubated for 1 h at room temperature. Afterwards, 200 µL of 15 nM PDDA was added into the solution and the solution was again incubated for 30 min. Finally, 600 µL of 5 nM AuNPs were added followed by the UV–vis measurements. Various pesticides such as atrazine, chlorosulfuron, 2,4-D, diuron and phorate were used to test the selectivity of the biosensor.

2.5. Determination of malathion in spiked samples

The practicability of the aptasensor was evaluated by performing the analysis in real samples i.e. food and water. The lake water was obtained from Sukhna Lake, Chandigarh, India and filtered using 0.4 µm filter in order to remove any suspended impurities. Subsequently, the lake water was spiked with different malathion concentrations followed by the analyses as described above. Apple was chosen as the matrix to test the feasibility of the biosensor in food. Firstly, the apple was cut and crushed into a homogenate followed by extraction with methanol. The sample was then filtered to remove the solid part and mixed with active charcoal to eliminate colored impurities. Finally, the solvent was evaporated and the residue was diluted with water. Malathion was then spiked in the samples and detected using the proposed assay.

2.6. Validation of the proposed assay with conventional technique

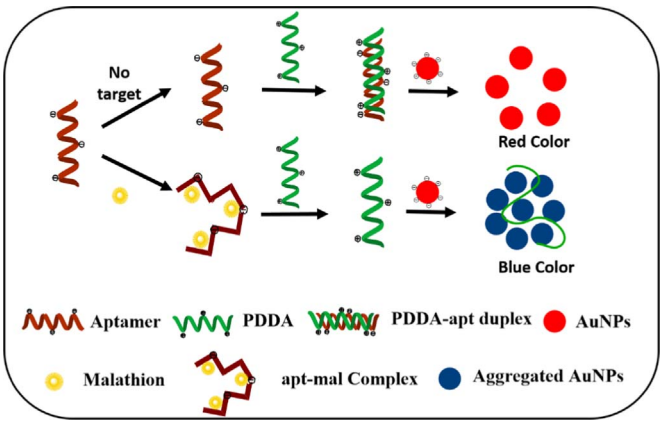
The performance of the aptasensor was validated with the conventional HPLC. For that, prior to use, all the malathion samples were filtered using 0.4 µm filter. Water-acetonitrile system was used as the mobile phase to carry out the analysis.

3. Results and discussion

The strategy for the colorimetric detection of malathion is illustrated in Scheme 1. In the absence of malathion, the aptamer is free and hybridize to form a duplex with the cationic PDDA owing to the interaction of negatively charged phosphate backbone of aptamer with PDDA. Thus, the aggregation of AuNPs is prevented due to the lack of sufficient PDDA. However, upon the addition of malathion, the aptamer forms a complex with malathion which, in turn, makes the PDDA free and results in the aggregation of AuNPs. Consequently, the remarkable change in the color of the AuNPs from red to blue is evident from naked eyes. The color of the solution is dependent on the concentration of PDDA which is directly linked to the concentration of malathion. Hence, the present methodology can be employed for detecting the presence of malathion colorimetrically.

3.1. Optimization of the reaction conditions

In order to develop a highly sensitive aptasensor for malathion,



Scheme 1. Schematic illustration of a colorimetric aptasensor based on gold nanoparticles for the detection of malathion. In the absence of malathion, the aptamer interacts only with the polymer and hence, the gold nanoparticles are well dispersed due to lack of sufficient amount of PDDA. However, in the presence of malathion, the aptamer interacts with the malathion and free PDDA aggregate the AuNPs, thereby leading to the color change of the solution from red to blue.