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Detection of organophosphorus pesticide – Malathion in environmental samples using peptide and aptamer based nanoprobes

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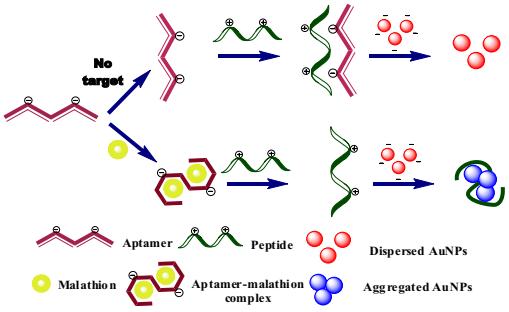
HIGHLIGHTS

- Highly sensitive, selective and rapid method is presented for detection of malathion.
- Method employs gold nanoparticles, aptamers and cationic peptide.
- Color of particles remains red in the absence of malathion and turn blue in its presence.
- Detection is also possible with naked eyes without requiring complex instrumentation.

GRAPHICAL ABSTRACT



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ABSTRACT

The contamination of environment with pesticides residues has necessitated the development of rapid, easy and highly sensitive approaches for the detection of pesticides. Thus, the prime objective of the present strategy was sensing of malathion, a toxic organophosphorus pesticide, widely used in agricultural fields, employing aptamer, cationic peptide and unmodified gold nanoparticles. The role of peptide was carefully evaluated to exploit its potential in colorimetric detection of pesticides. The peptide, when linked to the aptamer renders the gold nanoparticles free and therefore, red in color. However, when the aptamer is associated with malathion, the peptide remains available to cause the aggregation of the nanoparticles and turn the suspension blue. The methodology utilizes the optical changes of the gold nanoparticles for the colorimetric detection of malathion. The method was found to be linear in the range of 0.01–0.75 nM with a limit of detection as 1.94 pM which is significantly lower than the other available reports. Moreover, the sensitivity of the developed nanoparticle based peptide aptasensor was tested in real samples and the results implied the high practicability of the method. Therefore, the present approach may pave a way for the sensitive, yet simple detection of different analytes without the need of expensive instrumentation.

1. Introduction

Organophosphorus pesticides (OPs) represent the class of pesticides that are widely used in agricultural fields in order to avoid the crop losses arising due to variety of pests [1–3]. Due to their widespread usage, their persistence in environment has raised

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serious human health and environmental concerns [4]. The OPs are extremely toxic as they are potential inhibitors of cholinesterase, an enzyme crucial for the functioning of central and peripheral nervous system [5–7]. The inhibition of esterase may result in various clinical complications such as including respiratory tract, paralysis or even death [8]. Earlier reports have estimated that millions of tons of OPs are employed to control the pests worldwide and are responsible for approximately 200,000 deaths every year due to phosphate poisoning particularly in developing nations [9,10]. Thus, owing to their indispensable use the need for the fabrication of reliable, rapid, selective and highly sensitive assays for the onsite monitoring of toxic OPs is clearly evident.

Although, conventionally available techniques such as HPLC, Mass spectrometry offer high sensitivity, they suffer from numerous shortcomings such as their time consuming nature, high cost and requirement of advanced instrumentation severely limiting their on-site usage [11–13]. Additionally, several enzymatic and immunoassays methods have also been explored for the determination OPs using various optical or electrochemical methodologies [14–16]. While high selectivity and sensitivity can be achieved, their widespread use is still limited due to the poor stability of the enzymes or antibodies. Thus, a more cost effective method, where the need for sophisticated instrumentation is minimized, yet which can provide highly sensitive and accurate determination of pesticides residues is essential.

In the past few decades, colorimetric sensors have appeared as promising alternatives to conventional techniques for residual analyte detection due to their benefits like rapidity, simplicity, cost effectiveness etc. [17,18]. Recently, metallic nanoparticles have seen a great emergence as colorimetric reporters due to their surface plasmon resonance (SPR), versatile surface modification, and good biocompatibility [19]. Gold nanoparticles (AuNPs) serves as excellent scaffolds for designing novel chemical or biological sensors owing to their distinct physical and chemical attributes [20]. The different agglomeration states of AuNPs resulting in distinct color changes make AuNPs an ideal optical indicator for signaling analyte recognition events [21]. Inspite of the numerous advantages of the colorimetric sensors, the lack of specificity still remains a major challenge. In order to overcome this challenge aptamer based biosensors have gained substantial attention in various fields for instance biochemistry, analytical chemistry, and detection science [22]. Aptamers are short and single-stranded oligonucleotides integrated to biosensing technologies as biorecognition moieties in order to generate high selectivity to their target with strong specificity and affinity. Moreover, aptamers can be engineered for analytes ranging from small sizes to cells and exhibit significant advantages such as cheap and easy synthesis, lack of immunogenicity, high thermal stability, and reversible denaturation relative to antibodies [23,24]. The inherent advantages of aptamers biosensors like specificity, short analysis time, low cost, and little or no sample manipulation makes them extremely suitable for practical applications and biosensor development [25].

Keeping in view the environmental concern and challenges associated with the conventional strategies, the aim of the present study is to chemically engineer facile and sensitive biosensor for OPs using simpler molecules. In order to realize the potential of small peptides in replacement to large polymers in sensing, herein, we report a methodology for the detection of trace levels of malathion, a toxic OP using aptamer as bio recognition element, cationic peptide as aggregation inducer and AuNPs as optical indicators for signaling the interaction of malathion with aptamer. The role of peptide as a suitable aggregating agent was thoroughly studied. Since hexapeptides are known to possess specific conformations therefore, it was assumed that they might interact more effectively to aptamer as compared to other smaller peptides i.e. di/tripeptides and hence hexapeptide was selected for the whole experimentation. It is pertinent to mention here that the cationic peptide based colorimetric detection of pesticides is being reported for the first time though the detection has already been reported using polymer. In the absence of malathion, the random coil aptamer interacts with the cationic peptide owing to electrostatic interactions and hence, no peptide is available to cause the aggregation of particles. Thus, the AuNPs maintain their dispersity and remain red in color. However, in the presence of malathion, the aptamer binds to malathion and acquires a rigid conformation thereby rendering the peptide free. Consequently, the free peptide interacts with AuNPs and affect their aggregation, thus, turning the color of the solution blue. To the best of our knowledge, this is the first report that describes the detection of malathion using combination of aptamer, peptide and AuNPs. Moreover, direct visualization is possible without the aid of sophisticated instruments.

2. Experimental details

2.1. Materials and methods

Hydrogen tetrachloroaurate (III) trihydrate, trisodium citrate dihydrate, aptamer, malathion, Fmoc-Arg(Pbf)-OH, Fmoc-Lys (Boc)-OHH, dimethylformamide, *N*,*N*,*N*′,*N*′-Tetramethyl-*O*-(benzotriazol-1-yl)uronium tetrafluoroborate (TBTU), *N*,*N*-diisopropylethylamine (DIEA), piperidine, trifluoroacetic acid, diethyl ether triisopropylsilane and acetonitrile were acquired from Sigma Aldrich (India). The oligonucleotide having the sequence 5′ATCCGTCACACCTGCTCTTATACACAATTGTTTTTCTCTTA ACTTCTTGACTGCTGGTGTTGGCTCCCGTAT-3′, obtained from literature, was desalted and HPLC purified [26]. All the reagents used were of analytical grade and were used as obtained without further purifications. All the experiments were carried out using Milli-Q water having a resistivity of 18.2 MΩ cm. The glassware was rinsed with aqua regia prior to use.

2.2. Synthesis of cationic peptide (KRKRKR)

The peptide was synthesized using the standard fluorenylmethyloxycarbonyl (Fmoc) solid-phase peptide synthesis based strategy. Initially, Wang resin was employed followed by subsequent coupling steps using TBTU and DIEA. The intermediate Fmoc deprotection steps were carried out using 20% piperidine. The final deprotection step was performed using standard protocol (95% trifluoroacetic acid/2.5% triisopropylsilane/2.5% ELGA water) for 3 h at room temperature. The crude peptide was analyzed by reverse phase high performance liquid chromatography (RP-HPLC) on a C18 column and then characterized by matrix assisted laser deso rption-ionization/time-of-flight (MALDI-TOF) mass spectrometry.

2.3. Synthesis of gold nanoparticles

The synthesis of AuNPs was carried out by citrate reduction of chloroauric acid [27]. Briefly, an aqueous of 0.01% $HAuCl_4$ (100 mL) solution was heated to boiling followed by the rapid addition of 1% trisodium citrate solution (2 mL). Initially, the colorless solution turned pale yellow and finally became red indicating the formation of AuNPs. Further, the solution was boiled for an additional 10 min. and allowed to cool at room temperature under stirring. The purification of the resulting particles was done using centrifugation at 12,000 rpm. The obtained colloids were stored in dark bottles at 4 °C.

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