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A supersensitive silver nanoprobe based aptasensor for low cost detection of malathion residues in water and food samples

Rajni Bala^a, Sherry Mittal^a, Rohit K. Sharma^{a,*}, Nishima Wangoo^{b,*}^a Department of Chemistry, Centre for Advanced Studies in Chemistry, Panjab University, Sector-14, Chandigarh 160014, India^b Department of Applied Sciences, University Institute of Engineering & Technology (U.I.E.T.), Panjab University, Sector-25, Chandigarh 160014, India

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ABSTRACT

In the present study, we report a highly sensitive, rapid and low cost colorimetric monitoring of malathion (an organophosphate insecticide) employing a basic hexapeptide, malathion specific aptamer (oligonucleotide) and silver nanoparticles (AgNPs) as a nanoprobe. AgNPs are made to interact with the aptamer and peptide to give different optical responses depending upon the presence or absence of malathion. The nanoparticles remain yellow in color in the absence of malathion owing to the binding of aptamer with peptide which otherwise tends to aggregate the particles because of charge based interactions. In the presence of malathion, the agglomeration of the particles occurs which turns the solution orange. Furthermore, the developed aptasensor was successfully applied to detect malathion in various water samples and apple. The detection offered high recoveries in the range of 89–120% with the relative standard deviation within 2.98–4.78%. The proposed methodology exhibited excellent selectivity and a very low limit of detection i.e. 0.5 pM was achieved. The developed facile, rapid and low cost silver nanoprobe based on aptamer and peptide proved to be potentially applicable for highly selective and sensitive colorimetric sensing of trace levels of malathion in complex environmental samples.

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1. Introduction

Noble-metal nanoparticles have gained substantial prominence owing to their excellent optical properties arising from their strong size dependence. The properties of these nanoparticles are entirely different from bulk metal due to surface plasmon resonance (SPR) bands, characteristic of nanoparticles but absent in bulk metal [1–3]. The SPR bands result as a consequence of the resonance of the incident photon frequency with collective oscillations of the conduction electrons. In particular, the SPR band is known as localized surface plasmon resonance (LSPR) band for metal nanoparticles. The LSPR peak is greatly influenced by various parameters such as interparticle distance, surrounding environment, shape and size of the nanoparticles [4,5]. The modulation of these parameters can indeed be useful in designing novel strategies in varied applications. For instance, the color changes arising due to interparticle plasmon coupling has been exploited in colorimetric sensing of different analytes [6–8]. However, in spite of numerous benefits of colorimetric sensors such as low cost, non-requirement of trained personnel and sophisticated instrumentation, the lack of specificity limits their practical usage [9]. In recent years, various colorimetric sensors have been devised but most of the research has been focused towards gold nanoparticles (AuNPs) and very little

work has been directed towards other noble metal nanoparticles such as silver. This can be attributed to relatively easier synthesis of AuNPs in comparison to silver. However, silver nanoparticles (AgNPs) offer certain advantages as evident from the molar extinction coefficient of AgNPs which is 100 times greater than that of AuNPs and hence can result in increased sensitivity and improved visibility due to the difference in optical brightness [10]. So far, very few literature reports are available utilizing the optical properties of AgNPs in colorimetric sensing [11]. Owing to the low cost of silver as compared to gold, AgNPs can provide a promising economical alternative to the widely employed AuNPs in colorimetric sensing.

Since many years, a wide variety of pesticides are being employed in agriculture across the world to protect plants from insects and pests infestation [12]. However, their indispensable use has resulted in serious contamination of air, water and land, thereby posing threat to the environment including humans [13–15]. Among diverse classes of pesticides, organophosphorus pesticides (OPs) are the most extensively used pesticides especially in agriculture, medicine industry and chemical warfare agents (CWA) due to their low persistence as compared to other pesticides [16]. However, OPs are highly toxic owing to irreversible phosphorylation and inhibition of acetylcholinesterase (AChE), an essential serine enzyme crucial for the proper functioning of the peripheral and central nervous system [17]. Consequently, the inactivation of AChE may lead to perturbation of the nervous system and results in serious complications including acute toxicity, cholinergic dysfunction

* Corresponding authors.

E-mail address: nishima@pu.ac.in (N. Wangoo).

and rapid paralysis of vital functions in living beings [18]. Among different organophosphates, malathion is the most widely employed insecticide in agricultural as well as residential areas. Its adverse effects on human health include numbness, decreased coordination, dizziness, tremor, nausea, blurred vision, difficulty breathing, slow heartbeat, headache and tingling sensations. Malathion is slightly toxic to mammals, moderately toxic to birds, and highly toxic to aquatic organisms, both freshwater and estuarine, as well as bees. According to the IARC, it is classified into category 2A (probably carcinogenic to humans). Therefore, its detection even in trace amounts is very important from an environmental point of view. Currently, different conventional methods such as capillary electrophoresis, gas chromatography, liquid chromatography, and chromatographic techniques coupled to mass spectrometry are available for OPs detection [19–22]. Though these techniques provide reliable and sensitive results, they suffer from numerous shortcomings such as they are highly time consuming, expensive, require trained labor, sophisticated instrumentation and complex sample pretreatments, thereby limiting their use in onsite monitoring of pesticides. Recent years have seen a great emergence in enzyme/antibodies based methodologies in OPs detection since these biosensors appear to be a better alternative to the conventional techniques [23]. However, the generation and stability of enzymes/antibodies limits their applicability for infield measurements. Therefore, there is a huge practical interest in designing simple, rapid, cost effective yet sensitive approaches for the detection of widely used OPs.

Previously, our group focused on the design of colorimetric biosensors for various organophosphorus pesticides by employing AuNPs [7,24–26]. The present approach of using AgNPs emphasizes on two important aspects for fabricating a colorimetric aptasensor. The first aspect focuses on the thorough evaluation of AgNPs in order to fully exploit their potential as optical indicators in devising a novel biosensor for the widely used OP malathion. Secondly, the role of the hexapeptide KKKRRR has been carefully investigated to realize its efficiency in replacing larger polymers which are already well established in sensing. So far the use of peptides remains unexplored in sensing applications. The methodology is based on the difference in the color responses of AgNPs that result due to distinct interactions. The color of the particles remains yellow in the absence of malathion due to the electrostatic binding between an aptamer specific for malathion and the cationic hexapeptide KKKRRR which otherwise tends to aggregate AgNPs due to electrostatic interactions. However, in the presence of malathion, the strong affinity of the oligonucleotide towards malathion renders the peptide free which then brings about the aggregation of particles and turns them orange. Thus, the present study opens up new pathways for the utilization of new molecules such as peptides in fabricating novel sensors. To the best of our knowledge, this is the first report where AgNPs have been employed in the colorimetric detection of any kind of pesticide along with an aptamer and a peptide.

2. Experimental

2.1. Materials and Methods

Silver nitrate, trisodium citrate dihydrate, aptamer, malathion, Fmoc-Arg(Pbf)-OH, Fmoc-Lys(Boc)-OH, 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 obtained from Sigma Aldrich (India). The oligonucleotide (aptamer) having the sequence 5'ATCCGTCACACCTGCTCTTATACACAATTGTTTTCTCTAAC TTCTTGACTGCTGGTGTGGCTCCCGTAT-3' [25] was used as received. All the reagents used were of analytical grade and no further modifications were carried out before use. The experiments were performed in ultrapure water having a resistivity of 18.2 MΩ cm and molecular grade water. The glassware was thoroughly washed with aqua regia prior to use.

2.2. Silver Nanoparticles Synthesis

The synthesis of AgNPs was carried out by using sodium borohydride as reducing and trisodium citrate as capping agent. Briefly, 100 mL of aqueous solution containing 0.25 mM AgNO₃ and 0.5 mM trisodium citrate solution was stirred for a few min at room temperature. Afterwards, 5 mL of ice-cooled 0.05 M NaBH₄ was added to the solution. The color changed from colorless to yellow indicated the formation of AgNPs. The solution was further stirred for an additional 10 min and stored in dark bottles at 4 °C.

2.3. Synthesis of KKKRRR

The synthesis of the desired peptide was carried out using the standard fluorenylmethyloxycarbonyl (Fmoc) solid-phase peptide synthesis based strategy [25]. To begin with, 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 done using standard protocol (95% trifluoroacetic acid/2.5% triisopropylsilane/2.5% ELGA water) for 3 h at room temperature. Analysis of the crude peptide was performed using reverse phase high performance liquid chromatography (RP-HPLC) on a C18 column followed by characterization using matrix assisted laser desorption-ionization/time-of-flight (MALDI-TOF) mass spectrometry.

2.4. Analysis of Malathion

A stock solution of malathion (12 mM) was prepared in acetone and stored at 4 °C. Various dilutions of malathion were then prepared in phosphate buffer (pH = 7.32). For the detection of malathion, 10 μL of 500 nM aptamer was mixed with 70 μL of different malathion concentrations, diluted with 100 μL phosphate buffer and incubated for 40 min. at room temperature. Subsequently, 50 μL of 10 μM peptide was added into the solution and the solution was again incubated for 20 min. Finally, 500 μL of AgNPs (2 nM) were added followed by the UV-visible measurements. The selectivity of the biosensor was also evaluated in the presence of various pesticides (0.5 μM) such as atrazine, chlorsulfuron, chlorpyrifos, 2,4-D, diuron, ethion and phorate.

2.5. Analysis in Spiked Samples

In order to assess the applicability of the proposed aptasensor in real samples, the analysis were carried out in real samples i.e. food and water. The lake water was obtained from Sukhna Lake, Chandigarh, India and spiked with different malathion concentrations followed by the analysis as described above. Apple was selected as the matrix to test the viability of the biosensor in food matrices. Firstly, the apple was cut and crushed into a homogenate manually followed by extraction with methanol (10 times) at rt. Then the sample was filtered and the solvent was evaporated followed by dilution with water (10 times). Finally, malathion was spiked and samples were analyzed using the proposed assay.

Caution: Pesticides are hazardous to health therefore, all the safety measures were taken during the analysis.

3. Results and Discussion

3.1. Principle of the Detection

In the past few years, most of the colorimetric detection methodologies have been focused towards using AuNPs owing to its excellent optical properties. As a consequence of this, the role of AgNPs has remained largely unexplored in this field, although the cost of silver nanoparticles is far less than that of gold nanoparticles. In this view, the present methodology is based on distinct colorimetric responses of AgNPs and the mechanism for the colorimetric detection of malathion using unmodified silver nanoprobe is illustrated in Scheme 1.

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