Sensors and Actuators B 210 (2015) 425–430

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Highly sensitive colorimetric detection of ethyl parathion using gold nanoprobes

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ARTICLE INFO

Article history:
Received 14 August 2014
Received in revised form
10 December 2014
Accepted 21 December 2014
Available online 8 January 2015

Keywords:
Colorimetric
Gold nanoprobes
Ethyl parathion

ABSTRACT

This study reports a simple, rapid and sensitive method using cysteine capped gold nanoparticles as key material for the detection of highly toxic and widely used organophosphate, ethyl parathion. The detection is based on the aggregation of cysteine capped gold nanoparticles leading to a visible colour change from red to blue as a consequence of the generation of thiocholine (TCh). The hydrolytic reaction of acetylthiocholine (ATCh) by the enzyme acetylcholinesterase (AChE) affects the production of thiocholine (TCh). Presence of ethyl parathion leads to suppression of TCh resulting in no colour change whereas its absence leads to a visible colour change from red to blue. The changes in the absorbance of the gold nanoparticle probe solution can be used as a method to determine the amount of pesticide in a given sample. The developed biosensor is rapid and has a limit of detection of 0.081 ng mL^{-1}. This simple and facile methodology makes the method highly suitable for the detection of pesticides in general and organophosphates, in particular.

1. Introduction

The enormous use of pesticides over the past many years has now posed a serious threat to the ecosystem since pesticide residues may enter into the food chain through air, water and soil [1,2]. This has resulted in the detection of even trace quantities of pesticide residues. Among the various classes of pesticides, the use of organophosphate pesticides (OPs) is extensive and has replaced several organochlorine pesticides [3]. However, OPs are found to be quite toxic and their acute toxicity can be ascribed to their irreversible phosphorylation and inactivation of the enzyme acetylcholinesterase, an essential enzyme involved in the cholinergic functions, in the central and peripheral nervous system [4]. Consequently, OPs inhibit the hydrolysis of the neurotransmitter acetylcholine, resulting in the in vivo accretion of acetylcholine, which inflicts various clinical complications including respiratory tract, paralysis or even death [5].

The traditional methods for the detection of pesticides include high performance liquid chromatography (HPLC) and/or gas chromatography–mass spectrometry (GC–MS) [6,7]. Although these techniques offer powerful trace analysis coupled with high reproducibility and very low limit of detection, they are time-consuming, laborious, and require expensive equipments and highly trained personnel which limits their use for on-site screening. Various analytical devices such as fluorescent probes [8–10], electrochemical sensors [11–13], immunosensors [14–16] and fluorescent resonance energy transfer (FRET)–quantum dots (QDs) based sensors [17,18] have been developed to overcome these challenges in the past decade. However, many of these technologies involve complicated chemical and physical methodologies. In specific, immunosensors possess very high sensitivity, but their drawback lies in the generation of antibody which, in pure form, is quite complex and requires tiresome effort.

Over the last two decades, various nanomaterials such as carbon nanotubes, ZrO_2 nanoparticles, quantum dots (QDs) and gold nanoparticles (AuNPs) have been employed in biosensing, resulting in a dramatic enhancement in the sensitivity towards OPs detection [19,20]. The effective use of numerous surface plasmon resonance (SPR) based sensors [21] has made the analyte detection possible whereas trace determination has been achieved by the use of surface enhanced Raman spectroscopy (SERS) and enzyme based sensing [22–24]. Among all the nanoparticles, gold nanoparticles have been employed extensively, owing to their excellent optical properties, relative ease of preparation and convenient surface functionalization [25]. The colour of the AuNPs is red in their well dispersed state which changes to blue upon aggregation, a feature directly exploited in colorimetric sensing [26,27]. Various

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http://dx.doi.org/10.1016/j.snb.2014.12.123
0925-4005/

contaminants such as copper, mercury, etc. have been detected using AuNPs based on the colorimetric phenomenon [28–30]. Cysteine capped AuNPs have ascertained to be efficacious as colorimetric sensors for the detection of trinitrotoluene, copper etc. [31,32], but there are no existing reports for their use in pesticide detection till date.

Keeping the above stated facts in mind, in this study, we have established a simple, rapid, yet sufficiently sensitive method for the detection of highly toxic and widely used organophosphate, ethyl parathion. The detection follows the principle that aggregation of cysteine capped gold nanoparticles (Cys-AuNPs) imparts a colour change from red to blue, which results due to the generation of thiocholine (TCh) as illustrated in Scheme 1. The major product as a result of the hydrolytic reaction of acetylthiocholine (ATCh) by the enzyme acetylcholinesterase (AChE) is TCh. Signalling of ethyl parathion can be easily done as its presence impedes AChE activity, leading to the suppression of TCh and consequently no colour change of gold nanoparticles due to lack of aggregation. The changes are reflected in the absorbance spectra as well as in the colour of Cys-AuNPs. To the best of our knowledge, this is the first report in which cysteine capped gold nanoparticles have been employed as probes for the rapid and sensitive detection of ethyl parathion.

2. Experimental

2.1. Reagents and methods

2.1.1. Chemicals

L-Cysteine, S-acetylthiocholine chloride, acetylcholinesterase, hydrogen tetrachloroaurate (III) trihydrate, trisodium citrate dihydrate, dithiobisnitrobenzoate (DTNB) and ethyl parathion were purchased from Sigma–Aldrich (India). All the experiments were performed using Milli-Q ultrapure water and the reagents used were of analytical grade. The glassware was rinsed in aqua regia prior to use.

2.2. Gold nanoparticles synthesis

The spherical gold nanoparticles with a diameter of 13 nm were prepared using a modified reported method [33]. Briefly, $HAuCl_4{\cdot}3H_2O$ (100 mL, 0.25 mM) in water was heated to boiling under constant stirring followed by the rapid addition of trisodium citrate (2 mL, 34 mM). The formation of AuNPs was confirmed by the dark ruby red colour of the solution. Further, the solution was boiled for 10 min and then allowed to cool at room temperature (rt). The resulting gold nanoparticles were then characterized by using transmission electron microscope (TEM) (H-7500, Hitachi), FT-IR (Thermoscientific, Nicolet iS50 FT-IR) and UV-vis spectrophotometer (Jasco, V-530). The particle concentration was measured using a molar extinction coefficient of $2.4 \times 10^8\ M^{-1}\ cm^{-1}$ at 520 nm as reported in literature [34].

2.3. Surface modification of gold nanoparticles with cysteine

The synthesis of cysteine capped AuNPs was carried out by mixing L-cysteine (10^{-6} M) and AuNPs (3 nM) in a volume ratio of 1:9 [31]. The solution was then stirred overnight at room temperature. Excess of cysteine was removed by centrifugation at 8500 rpm for 20 min. The synthesized Cys-AuNPs were then characterized using FT-IR, thermogravimetric analysis (TGA) (SDT Q 600, TA), TEM and UV–vis spectroscopy.

2.4. Preparation of stock solution of ethyl parathion

Ethyl parathion (3.4 mg) was dissolved in methanol (1 mL) to prepare its stock solution and stored at 0 °C. Different dilutions of ethyl parathion were freshly prepared in methanol and used for further experiments.

2.5. Colorimetric assay for pesticide detection

To test the performance of Cys-AuNPs as a colorimetric probe in pesticide detection, different concentrations of ethyl parathion (50 μL) were added to AChE (10 μL, 200 mU mL^{-1}) and the resulting solutions were incubated for 2.5 h at rt. Then, ATCh (100 μL, 15 μM) was added to the mixture and again incubated for 30 min. Finally, Cys-AuNPs (0.9 mL, 5 nM) were added and the absorption was measured for Cys-AuNPs at 520 nm (A_{520}).

2.6. Measurement of inhibition of enzyme activity

Inhibition of AChE activity by ethyl parathion was determined spectrophotometrically using DTNB [35]. Ethyl parathion (50 μL) of variable concentration was added to AChE (400 μL, 49.5 mU mL^{-1}). The mixtures were incubated for 2 h at rt followed by the addition of S-acetylthiocholine chloride (20 μL, 10 mM) and chromogen DTNB (20 μL, 10 mM).

2.7. Analysis of ethyl parathion in spiked water

The feasibility of the above method was tested by performing the analysis using spiked tap water, mineral water and lake water. The spiked samples (50 μL) were mixed with AChE (200 mU mL^{-1}) and incubated for 2.5 h. The mixture was further incubated for 30 min after the addition of ATCh (100 μL, 15 μM) and Cys-AuNPs followed by the colorimetric measurements. Ethyl parathion residue was then determined using the standard correlation curve.

3. Results and discussion

3.1. Surface functionalization of AuNPs

The most commonly used reducing agent for the synthesis of gold nanoparticles is trisodium citrate, but the weak electrostatic interactions between citrate and AuNPs is not been considered ideal for detection as well as sensing purposes. Therefore, very few literature reports are available utilizing cit-AuNPs in colorimetric assays [36]. To overcome this problem, the gold surface was modified with an amino acid namely cysteine via gold–sulfur (Au–S) linkage. As compared to citrate ions, the thiol moiety of cysteine is known to have much stronger interaction with the Au surface [37–39].

To synthesize Cys-AuNPs, the AuNPs were first prepared using citrate as the reducing agent. The unique surface plasmon peak of cit-AuNPs at 520 nm can be attributed to the monodispersity of the particles (see Fig. S1 in Supplementary data). The surface functionalization of cit-AuNPs with cysteine was later performed by the addition of L-cysteine. The colour of solution remained the same upon the addition of cysteine, which indicated that within this volume ratio, no aggregation of particles occurred. The Cys-AuNPs were found to be quite stable, mainly due to strong Au-thiol linkage. The thermogravimetric studies further established the stability and capping of the AuNPs (see Fig. S2 in Supplementary data). The characterization of Cys-AuNPs was further done using FT-IR spectroscopy. The FT-IR spectrum displayed a sharp peak at 2550 cm^{-1} which corresponds to the S–H stretching (see Fig. S3 in Supplementary data). Peaks at 1575 cm^{-1} and 1390 cm^{-1} accounted to the asymmetric and symmetric stretching of COO^- group, respectively. Further, N–H bending was observed at 1525 cm^{-1} and a broad band