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A novel fluorimetric sensing platform for highly sensitive detection of organophosphorus pesticides by using egg white-encapsulated gold nanoclusters

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ABSTRACT

Assays for organophosphorus pesticides (OPs) with high sensitivity as well as on-site screening have been urgently required to protect ecosystem and prevent disease. Herein, a novel fluorimetric sensing platform was constructed for quantitative detection of OPs via tyrosinase (TYR) enzyme-controlled quenching of gold nanoclusters (AuNCs). One-step green synthetic approach was developed for the synthesis of AuNCs by using chicken egg white (CEW) as template and stabilizer. Initially, TYR can catalyze the oxidation of dopamine to dopaminechrome, which can efficiently quench the fluorescence intensity of AuNCs at 630 nm based on dynamic quenching process. However, with the presence of OPs, the activity of TYR was inhibited, resulting in the fluorescence recovery of AuNCs. This proposed fluorescence platform was demonstrated to enable rapid detection for OPs (paraoxon as model) and to provide excellent sensitivity with a detection limit of 0.1 ng mL^{-1}. Significantly, the fluorescence probe was used to prepare paper-based test strips for visual detection of OPs, which validated the excellent potential for real-time and on-site application.

1. Introduction

Organophosphorus pesticides (OPs) are popular substances utilized in agriculture across the world due to their high effectiveness for protecting crops (Zhang et al., 2010; Zheng et al., 2011a, 2011b; Liu et al., 2012). However, the extensive use and improper disposal of OPs could cause serious contamination of food, environment and ecosystem (Gao et al., 2012; Yan et al., 2015a). Unfortunately, the OPs residues could be hazardous to human health, which consequently result in impeded neurotransmission even at very low concentrations (Meng et al., 2013; Zhang et al., 2014; Chow et al., 2015; Kim et al., 2015; Qian and Lin, 2015). Thus, the development of a highly sensitive method for OPs detection is an urgent demand to protect the ecosystem, control food quality and safety, estimate OPs poisoning and prevent disease. Vast endeavors have been undertaken to develop various strategies for OPs detection, including chromatographic (Huang et al., 2002; Zacharis et al., 2012), electrochemical assay (Jha and Ramaprabhu, 2010; Du et al., 2011; Zhang et al., 2012), enzyme linked immunosorbent assays (ELISA, Yan et al., 2014) and colorimetric assays (Kim et al., 2015). However, conventional instrument-based methods require sophisticated instrumentation, tedious sample preparation and purification procedures. Electrochemical assay need time-consuming for complicated electrode labelling and modification procedures. Colorimetric assays suffer from low detection sensitivity and false-positive effect. ELISAs are susceptible to interferences from matrix components and also need costly bio-molecular reagents. Thus, these drawbacks obvious limited their on-site and real-time detection in most settings, particularly emergency cases (Long et al., 2015). Functional materials based fluorimetric assays have been employed as promising optical candidates for the quantifying OPs (Meng et al., 2013; Yi et al., 2013; Li et al., 2016). Recently, our group integrated dual-emission fluorescence probe (Yan et al., 2015b) for OPs monitoring through the change of ratiometric fluorescent intensity. The ratiometric fluorescent probe was designed by hybridizing two differently colored CdTe quantum dots (QDs), which not only contains toxic elements (Cd^{2+}), but also need complicated preparation process. Thus, the design of environmentally-friendly and facile synthesis sensing nanomaterial as well as on-site detection of OPs are the focal point of our research.

Noble metal nanoclusters which possess remarkable properties as well as facile synthetic routes have received significant investigative attention (Tao et al., 2015). Compared with other types of luminescent

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materials (Liao et al., 2013; Meng et al., 2013; Wang et al., 2015; Li et al., 2016), metal nanoclusters displayed distinct advantages, such as favorable water solubility, low toxicity and excellent biocompatibility (Cui et al., 2014). Especially, their one-step green synthesis without the requirement of toxic organic solvents makes metal nanoclusters widely attractive (Xie et al., 2009). Compared with silver and copper nanoclusters, the facile synthetic routes reported by Xie et al. (2009) of gold nanoclusters (AuNCs) do not need reducing agent (hydrazine hydrate and sodium borohydride) get more attention and more in-depth development. AuNCs has proved to be promising candidates in fabrication of fluorescent probes for the recognition of chemical species and imaging of cells (Liu et al., 2013). Several proteins (horseradish peroxidase, bovine serum albumin, trypsin, and so on) have been employed for synthesizing AuNCs (Xie et al., 2009; Wen et al., 2011; Sun et al., 2015). Recently, natural source biomass worked as chelating and reducing agents provided new inspiration of designing nanomaterials with valuable optical properties and less environmental impact. In this paper, chicken egg white (CEW), which contains rich proteins, were used as the candidates for preparing AuNCs.

Herein we designed a simple and rapid fluorimetric platform for the sensitive detection of OPs (paraoxon, as a model analyte) based on the AuNCs-tyrosinase (TYR) fluorescent probe. CEW as template and stabilizer was used to synthesize AuNCs, which exhibited the maximal emission at 630 nm. The produced dopaminechrome by TYR oxidation could effectively quench the fluorescence (FL) of AuNCs through the dynamic quenching process. OPs could efficiently suppress the activity of TYR (Yan et al., 2015b; Hou et al., 2015), resulting in the FL recovery of AuNCs. The proposed platform which combined the oxidation ability of TYR and the OPs-caused activity inhibition is specific to OPs and allows the detection limit of paraoxon down to 0.1 ng mL^{-1}. More importantly, the AuNCs can be successfully facilitated on a filter paper as test strips for rapid and visual detection of OPs.

2. Experiment

2.1. Reagents and instruments

Tyrosinase (TYR), dopamine (DA), paraoxon and hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4 \cdot xH_2O$) were used directly without further treatment. The water used throughout this study had a good resistivity (> 18 MΩ cm^{-1}). The FL spectra and ultraviolet spectra were collected by a RF-5301 PC spectrofluorophotometer (Japan) and Shimadzu UV-1700 UV–vis spectrophotometer (Japan). Transmission electron microscopy (TEM) was tested on a Philips Tecnai F20 TEM operating.

2.2. Synthesis of gold nanoclusters (AuNCs)

Gold nanoclusters were synthesized with chicken egg white (CEW) as template according to previously study (Josepha and Geckeler, 2014). All glassware was soaked with Aqua Regia (HCl:HNO_3=3:1, v/v) overnight, and then washed with ultrapure water. 5.0 mL of $HAuCl_4$ solution (10 mmol L^{-1}) and 5.0 mL of CEW solution (50 mg mL^{-1}) were mixed under vigorous stirring for 5 min at 37 °C. Then, 0.45 mL of NaOH solution (1 mol L^{-1}) was introduced into the mixture solution. The reaction was taken in 37 °C for 12 h. According to Chen et al. (2013), the concentration of AuNCs was estimated to be 4.78 mmol L^{-1}.

2.3. Procedures for OPs detection

100 μL different concentration of paraoxon were introduced to TRY solution in a 2.0 mL calibrated test tube for 25 min under room temperature. Then, 100 μL AuNCs and 200 μL DA (2 mmol L^{-1}) were successively introduced into the system. The solution was diluted to the mark with 10.0 mmol L^{-1} of PBS buffer (pH=7.0) and mixed thoroughly for 5 min at room temperature. The FL spectra were recorded for the detection of paraoxon.

2.4. Paper-based test strips

The AuNCs based test strips were prepared in a facile method. Briefly, filter paper (0.5 cm×0.5 cm) were soaked in 10 mL AuNCs solution (4.78 mmol L^{-1}) for 4 h. After drying in room temperature, 10 μL of reaction solution (the mixture of TYR and DA with various concentration of paraoxon) was drop on the surface of test papers. The test strips were placed under UV light (365 nm) for paraoxon detection.

3. Results and discussion

3.1. Characteristics of AuNCs

The fluorescent AuNCs were facilely synthesized by using CEW as template and stabilizer. CEW contains rich source of proteins which make it suitable for the preparation of AuNCs (Selvaprakash and Chen, 2014). The morphology of obtained AuNCs was characterized by employing TEM. As displayed in Fig. 1A, AuNCs were well dispersed and revealed the spherical morphology with the average diameter around 2–3 nm. Fig. 1B showed the as-prepared AuNCs exhibited the maximal emission at 630 nm (red line) under the excitation at 470 nm (black line), confirming the fluorescent feature of AuNCs. Inset in Fig. 1B showed the photographic images of AuNCs under sunlight (light yellow) and under the UV lamp with 365 nm (red). As shown in Fig. 1C, the UV–vis absorption spectrum of AuNCs was different from CEW in the wavelength ranging 250–400 nm. These results indicated that CEW-encapsulated AuNCs with bright fluorescent were successfully obtained using common CEW as precursor. The stability of as-prepared AuNCs was also systematically investigated after irradiating continuously over a period of 60 min (Fig. 1D). It can be seen that the FL intensity of AuNCs remains stable with no obvious change, implying that the AuNCs had a high stability and could be applied for fabrication sensor.

3.2. The detection strategy of OPs

In order to construct a sensitive platform for OPs detection, the AuNCs-based sensing system combined the enzymatic reaction of TYR and the inhibition ability of OPs was fabricated. AuNCs had an apparent emission peak at 630 nm, which could not be influenced by TYR or dopamine (DA) separately (Fig. S1). Under catalysis of TYR, DA was oxidized into dopaminechrome which can serve as a good electron acceptor. This process was also confirmed by UV–vis spectroscopy (Fig. S2). Dopaminechrome can efficiently quench the FL of AuNCs through the dynamic quenching process (Red line, Fig. 2A). OPs can suppress TYR activity and then result in FL recover of the AuNCs (Blue line, Fig. 2A), which provided the basis for FL detection of OPs.

According to previous research, dopaminechrome were reported as an efficient FL quencher for QDs and carbon dots (Li et al., 2014; Ma et al., 2015). For further explanation of the mechanism of FL quenching of AuNCs, we measured the zeta potential of AuNCs/TYR and AuNCs/TYR/DA. The zeta potential showed a significant reduction from −27.9 mV (AuNCs/TYR) to −18.8 mV (AuNCs/TYR/DA), suggesting that dopaminechrome has strong electrostatic interaction with AuNCs. Furthermore, the FL lifetime of AuNCs/TYR system in the presence and absence of DA were studied (Fig. 2**B**). The three decay components and their ratios for the FL lifetime of AuNCs/TYR and AuNCs/TYR/DA were shown in Table S1. The FL lifetime of AuNCs/TYR/DA (0.72 μs) was shorter than that of AuNCs/TYR (1.99 μs), implying that the dynamic quenching process was dominant for quenching mechanism (Tao et al., 2013; Teng et al., 2015). Additionally, static and dynamic quenching can also be distinguished