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Analytical Methods

Development of an upconversion fluorescence DNA probe for the detection of acetamiprid by magnetic nanoparticles separation

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ABSTRACT

An upconversion fluorescence DNA probe which consists of aptamer-conjugated magnet nanoparticles (apt-MNPs) and complementary DNA-conjugated upconversion nanoparticles (cDNA-UCNPs) was developed to detect acetamiprid. Acetamiprid can specifically conjugate with the apt-MNPs to dissociate the cDNA-UCNPs from the apt-MNPs and resulted in reduced fluorescence intensity through an external magnet. The change of fluorescence intensity (△I) is positively related to the concentration of acetamiprid, which can be applied for the quantification of acetamiprid. Under optimal conditions, a linear detection range and detection limit are 0.89–114.18 μg/L and 0.65 μg/L, respectively. The probe was successfully used to detect acetamiprid in spiked paddy water, soil, pear, apple, wheat and cucumber. Average recoveries are 78.2%–103.5% with intra-day relative standard deviations (RSDs) of 2.6%–10.9% and inter-day RSDs of 4.3%–10.2%. The amounts of acetamiprid in the authentic paddy water and pear samples detected by the DNA probe are significantly correlated with that detected by high-performance liquid chromatography (HPLC).

1. Introduction

Acetamiprid, a new broad-spectrum neonicotinoid insecticide, plays a key role in controlling sucking-type insects by interfering with insects' nervous system (Fan, Zhao, Shi, Liu, & Li, 2013; Fogel, Schneider, Desneux, Gonzalez, & Ronco, 2013; Shi, Zhao, Liu, Fan, & Cao, 2013). Many studies have proved that excessive residual acetamiprid can destroy ecosystem and harm human's health (Fan et al., 2013; Hu, Chen, Li, Ouyang, & Zhao, 2016; Liu, Su et al., 2016, Liu, Li et al., 2016; Rapini, Cincinelli, & Marrazza, 2016). Hence, it is vital to detect its residue in environment and agricultural products. At present, analyses of acetamiprid mainly depend on instrumental detections which usually are expensive cost, complex sample pretreatment and skilled technique (Lee et al., 2017). Therefore, it is necessary to develop an economical, simple and rapid method for the detection of residual acetamiprid.

DNA probes have been widely applied in disease diagnosis, cell therapy and drug delivery with high specificity and sensitivity. In general, DNA probes mainly contain recognition element and marker. Aptamers are single-stranded DNA or RNA (mainly DNA) sequences which are isolated in vitro from random-sequence nucleic acids libraries via the process of systematic evolution of ligands by exponential enrichment (SELEX) (Huo et al., 2016; Lin et al., 2016). They are able to bind with various cognate targets selectively and specifically through the change of configuration (Nutiu & Li, 2003; Nutiu & Li, 2005) which can be used as recognition elements in biological probe. Furthermore, aptamers are easily synthesized and modified, and have the advantages of good stability during long-time storage and cost-effectiveness, which have been expected to become a powerful tool for the detection of various chemical molecules. In recent years, aptamers have been successfully applied to detect small molecular compounds as recognition probes in biodetections (Danesh, Ramezani, Emrani, Abnous, & Taghdisi, 2016; Li et al., 2016; Lu, Chen, Wang, Zheng, & Li, 2015; Xiang & Tang, 2017).

Upconversion nanoparticles (UCNPs) have the property to emit visible light by the excitation of multiple low-energy near-infrared (NIR) photons, which attract more and more attention (Hu et al., 2016; Lin et al., 2012). In recent years, UCNPs have been widely applied as the fluorescent material for the biological probe due to their advantages in biological safety and optical property. Compared with conventional organic fluorophores and inorganic quantum dots (QDs), UCNPs possess several biological safety advantages, such as low toxicity, high chemical stability, and strong penetrability in living tissues (Han, Li, Huang, & Kong, 2016; Jiang & Meng, 2013; Wang & Liu, 2009; Wang et al., 2014; Xu et al., 2014; Yuan et al., 2013; Zhang et al., 2018). UCNPs have the optical properties of long fluorescence lifetimes, low photobleaching, high quantum yields, narrow emission peak, and large stokes shifts

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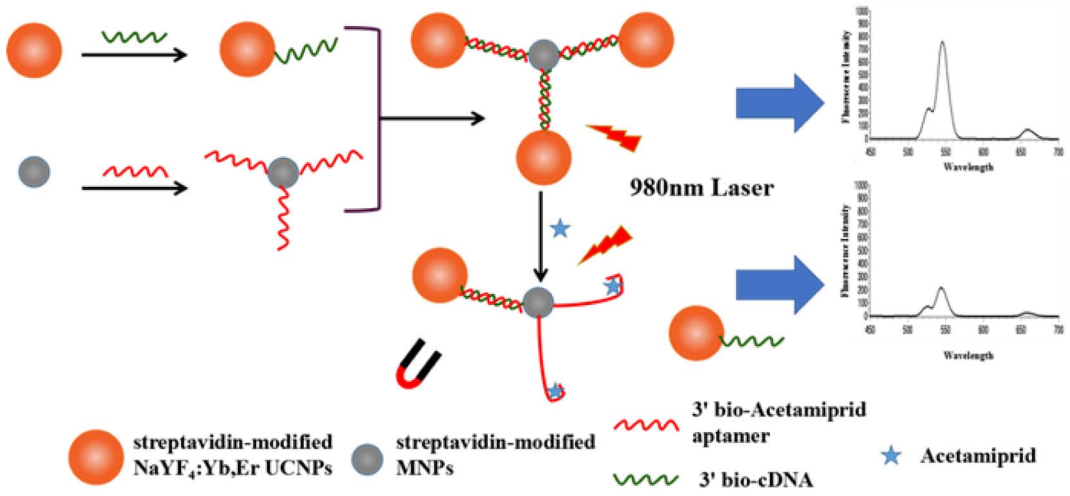

Fig. 1. Schematic illustration of the strategy to form the upconversion fluorescence DNA probe.

(Long, Li, Zhang, & Yao, 2015). Besides, the UCNPs are less disturbed by the autofluorescence resulting from background sample matrix because of the excitation by NIR (Jiang & Meng, 2013; Tsang, Chan, Wong, & Hao, 2015; Wu, Cao, Zhang, & Wang, 2018). All of these features make UCNPs a suitable fluorescence material in fluorescence probe.

In this study, an upconversion fluorescence DNA probe was developed for the detection of acetamiprid in environmental samples and agricultural products. The DNA probe was formed with the aptamer-conjugated MNPs (apt-MNPs) and complementary DNA-conjugated UCNPs (cDNA-UCNPs), so that it had the advantages of magnetic separation and concentration effect of the MNPs and the high sensitivity of the UCNPs. The sensitivity, specificity, precision and accuracy of the DNA probe were studied in detail through the optimization, cross-reactivity, and analysis of spiked and authentic samples.

2. Materials and methods

2.1. Materials

Streptavidin (99%) and acetamiprid (99%) were purchased from Aladdin Industrial Corporation (Shanghai, China). 25% glutaraldehyde solution was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The sequence of 20-mer acetamiprid-binding aptamer: 5′-CTGAC ACCAT ATTAT GAAGA-biotin-3′ and the sequence of its complementary DNA (cDNA): 5′-TCTTC ATAAT ATGGT GTCAG-biotin-3′ (He, Liu, Fan, & Liu, 2011) were synthesized by Sangon Biological Engineering Technology & Co., Ltd. (Shanghai, China). All the other chemicals used in this study were of analytical grade.

2.2. Apparatus

The morphology of MNPs and UCNPs was detected by an H-7650 transmission electron microscope (Hitachi, Japan). Fluorescence intensity was determined using F-2700 fluorescence photometer (Hitachi, Japan) with an external 980 nm laser source (Changchun Laser Optoelectronics Technology Co., Ltd., Changchun, China). The Fourier transform infrared spectrum (FTIR) of MNPs and UCNPs was determined by Vector-22 FTIR spectrophotometer (Bruker, Germany). The phase composition of amine-functionalized MNPs and UCNPs were determined by D8 Advance X-ray diffraction (Bruker, Germany).

2.3. Preparation of aptamer and cDNA-conjugated nanoparticles

The amino-modified MNPs (Hua et al., 2017) and amino-functionalized silica-capped $NaYF_4$:Yb, Er UCNPs (You et al., 2017) were prepared as previously described and stored in the laboratory. The amine-functionalized MNPs and UCNPs were both conjugated with streptavidin respectively by a classical glutaraldehyde method (Liu, Su et al., 2016, Liu, Li et al., 2016; Wu et al., 2015). Typically, 10 mg of the MNPs was dispersed in 5 mL of 0.14 M phosphate buffer solution (PBS) at pH 7.4 by ultrasonication for 15 min, and then 1.25 mL of 25% glutaraldehyde were quickly added and adequately distributed. The mixture was shaken in darkness for 2 h at room temperature. The MNPs were washed three times with PBS to remove the redundant glutaraldehyde through magnetically separating. The resultant MNPs were redispersed in 5 mL of PBS by ultrasonication and mixed with 100 μL of 1 mg/mL streptavidin. The mixture was shaken slowly at room temperature overnight. The streptavidin-conjugated MNPs were separated via an external magnet, and washed with PBS for several times. The streptavidin-activated MNPs were suspended in 5 mL PBS, followed by addition of 250 μL 1 μM biotin-modified aptamers, and then the mixture was incubated for 12 h under gentle shaking. After washing three times, the obtained apt-MNPs were dispersed in 5 mL of PBS and stored at 4 °C. The cDNA-UCNPs were prepared by using the same procedure of apt-MNPs, except for the separation of the UCNPs from the mixture by centrifugation at 4000 rpm for 5 min.

2.4. Analytical procedure

The paddy water samples were directly detected by the DNA probe after mixing with isometric 2× optimal buffer. Ten grams of solid samples were extracted by 20 mL of 20% methanol-PBS with 3 min homogenization and 15 min sonication. After centrifugation at 4000 rpm for 5 min, the supernatant was transferred into 25 mL volumetric flask and adjusted with PBS. After appropriate dilution, the samples were detected by the DNA probe.

As shown in Fig. 1, 220 μL cDNA-UCNPs and 60 μL apt-MNPs were incubated in a 1 mL centrifuge tube with 220 μL 0.1 M PBS at 37 °C for 50 min. The unbound cDNA-UCNPs were washed off thoroughly using PBS with an external magnet, and the cDNA-UCNPs/apt-MNPs hybridization probe was obtained. Subsequently, 250 μL of the standard solution of acetamiprid or sample solution were added to the hybridization probe. After interaction for 30 min, the MNPs were washed gently with PBS for several times and re-suspended in 500 μL PBS to detect the fluorescence intensity at excitation/emission wavelengths of 980/544 nm.