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Ultrasensitive detection of deltamethrin by immune magnetic nanoparticles separation coupled with surface plasmon resonance sensor

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ABSTRACT

Small molecules or analytes present in trace level are difficult to be detected directly using conventional surface plasmon resonance (SPR) sensor, due to its small changes in the refractive index induced by the binding of these analytes on the sensor surface. In this paper, a new approach that combines SPR sensor technology with Fe_3O_4 magnetic nanoparticles (MNPs) assays is developed for directly detecting of deltamethrin in soybean. The Fe_3O_4 MNPs conjugated with antibodies specific to antigen serves as both labels for enhancing refractive index change due to the capture of target analyte, and "vehicles" for the rapid delivery of analyte from a sample solution to the sensor surface. Meanwhile, SPR direct detection format without Fe_3O_4 MNPs and gas chromatography (GC) analysis were conducted for detection of deltamethrin in soybean to demonstrate the amplification effect of Fe_3O_4 MNPs. A good linear relationship was obtained between SPR responses and deltamethrin concentrations over a range of 0.01–1 ng/mL with the lowest measurable concentration of 0.01 ng/mL. The results reveal that the detection sensitivity for deltamethrin was improved by 4 orders of magnitude compared with SPR direct detection format. The recovery of 95.5–119.8% was obtained in soybean. The excellent selectivity of the present biosensor is also confirmed by two kinds of pesticides (fenvalerate and atrazine) as controls. This magnetic separation and amplification strategy has great potential for detection of other small analytes in trace level concentration, with high selectivity and sensitivity by altering the target-analyte-capture agent labeled to the carboxyl-coated Fe_3O_4 MNPs.

1. Introduction

As one of the most popular and widely used insecticides in the world (Elliot et al., 1974; Gorge and Nagel, 1990), deltamethrin belongs to the synthetic type II pyrethroid insecticide that kills insects through dermal contact and digestion. There are many uses for deltamethrin from agricultural uses to home pest control. Deltamethrin's popularity originates in its stability and longer residual activity, so residues are commonly found in food, consumer products and the environment (Koprücü and Aydın, 2004). Human exposure to deltamethrin can occur through inhalation, ingestion, and the dermal routes of eye and skin contact, each of those can possibly lead to acute health effects such as choreoathetosis, hyperexcitability, ataxia, dermatitis, diarrhea, tremors, and vomiting (Ray et al., 2000). Deltamethrin is also highly toxic as a neurotoxin to aquatic life, particularly fish (Velíšek et al., 2007). Consequently, it is very important to develop a rapid, high sensitive and specific detection method for monitoring deltamethrin residues in crop, drinking water and soil.

The traditional analytical methods for deltamethrin detection focus on the combination of chromatographic techniques and multiple detection techniques, such as gas chromatography with an electron capture detector (GC-ECD) (Ding et al., 2000) gas chromatography–mass spectrometry (GC–MS) (Esteve-Turrillas et al., 2005), high-performance liquid chromatography-UV detection (HPLC-UV) (Wongsa and Burakham, 2012), high-performance liquid chromatography-diode array detection (HPLC-DA) (Tsochatzis et al., 2010), high-performance liquid chromatography–mass spectrometry (HPLC–MS) (Ferrer et al., 2005), or liquid chromatography combined with postcolumn photochemically induced fluorometry derivatization and fluorescence detection (HPLC-FD) (Vázquez et al., 2008). Although those methods can give reproducible, reliable, accurate and sensitive

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determination results, they need costly apparatus, tedious and complicated multi-step sample preparation, which is time-consuming and ungreen.

With the development of various sensors and their combination with metal nanoparticles (NPs) or quantum dots, currently, the techniques has been progressed from the biomolecule detection to the small molecules detection. For example, sensor has had analytical performance as excellent as HPLC for deltamethrin detection (Ge et al., 2011a, 2011b). Among them, surface plasmon resonance (SPR) sensor, cooperating with nanoparticles as an amplification reagent, has been reported for the detection of small molecules and attracted more and more attention (Chang et al., 2011; Kim et al., 2012; Pelossof et al., 2012; Zamfir et al., 2011; J.L. Wang et al., 2011, Y. Wang et al., 2011). All kinds of NPs, including Au NPs (Urusov et al., 2011), Ag NPs (Vasileva et al., 2011), SiO_2 NPs (Luckarift et al., 2007), Pd NPs (Lin et al., 2008), and Pt NPs (Beccati et al., 2005), had been demonstrated for SPR signal amplification in small molecule detection. Although these methods can enhance effectively the SPR sensitivity enhancement, they usually require more time and complex process for the selective enrichment and separation of target molecules from a sample solution, especially for analytes in complex agriculture products or food matrixes (Liang et al., 2012).

Recently, magnetic nanoparticles (MNPs) have been received increasing attention on application for the immobilization and purification of biomolecules in SPR. There are three reasons for MNPs application in SPR: firstly, MNPs have the large surface area, which allows for a high density of biomolecule immobilization; secondly, MNPs have excellent magnetism, which allows for direct capture, separation, and even concentration of target molecules by external magnetic field (Chen et al., 2008; Heidari et al., 2012); thirdly, MNPs have high refractive index and the high molecular weight, which may effectively increase the SPR signal. These advantages make MNPs act not only as an amplifier to enhancing the sensitivity of the SPR sensor, but also as a concentration purification agent to reduce the background interference of unknown compound in SPR assay. In addition, MNPs are more economical than Au NPs, because of the low cost of Fe salts. So far, several research groups have confirmed superior performance of MNPs and demonstrated promising SPR biosensor applications for biomolecules detection. For example, J.L. Wang et al. (2010, 2011) have developed a novel SPR biosensor for detecting adenosine and thrombin based on indirect competitive inhibition assay and sandwich assay, respectively, using Fe_3O_4 MNP–aptamer conjugates as the amplification reagent. Liang et al. (2012) applied sandwich immunoassay based on the SPR biosensor to detect α-fetoprotein (AFP) by immobilizing a primary AFP antibody on the surface of a-mercapto-1-propanesulfonate/chitosan–ferrocene/Au NP film, employing Fe_3O_4@Au–AFP secondary antibody conjugates as the amplification reagent.

In this work, we report a new approach that combines SPR sensor technique with Fe_3O_4 MNP assay for simple, rapid and ultrasensitive detection of the deltamethrin. Here, the surface of the Fe_3O_4 MNPs was modified by carboxyl groups which make the MNPs be easily functionalized by antibodies. The Fe_3O_4 MNPs conjugated with antibodies simultaneously served as "vehicles" for rapid delivery of target analyte from a sample to the sensor surface, and served as labels increasing the SPR detection sensitivity due to high refractive index and the high molecular weight of Fe_3O_4 MNPs. Accordingly, the direct detection of deltamethrin can be achieved by injecting conjugates of the Fe_3O_4 MNP–anti-deltamethrin monoclonal antibody (Ab) enriched deltamethrin on the SPR sensor surface modified with chitosan (Scheme S1). Moreover, this assay can simplify the pretreatment process of the sample, and increase the detection accuracy of small deltamethrin in complex agriculture product or food matrixes. To demonstrate the usefulness of the approach developed, deltamethrin in soybean samples were analyzed with the SPR sensor, and the performance for deltamethrin analysis on the SPR sensor was further compared with that on SPR direct detection format and GC-ECD method, respectively. This proposed approach can carry out an accurate and ultrasensitive detection for deltamethrin, and also can be used to detect other analytes of interest by altering the corresponding antibody in the MNPs conjugates.

2. Experimental

2.1. Materials and reagents

Ab was obtained from WuHan SanYing Proteintech Group, Inc. Nodium hydroxide (NaOH), anhydrous ether, ethyl acetate, n-hexane and bovine serum albumin (BSA) were obtained from Beijing Dingguo Biotechnology (Beijing, China). Deltamethrin, 3-mercaptopropionic acid (MPA), n-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), mercaptoethylamine (MEA), chitosan (medium molecular weight) and ethanol amine (EA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All reagents were of analytical grade and used without further purification, and ultrapure water was used throughout this work.

Ab was dissolved with 0.01 M phosphate buffer solution (PBS, pH 7.4), and stored at 4 °C. Deltamethrin was dissolved with anhydrous ethanol as stock solution, and then diluted with PBS as standard solution. Blocking buffer solution was composed of PBS containing 0.1 M EA.

2.2. Apparatus

The SPR measurements were conducted on a BI-SPR 2000 system (Biosensing Instrument Inc., Tempe, AZ). A flow delivery system incorporated in the BI-SPR platform pumped samples onto the SPR sensor chip at a flow rate of 10 μL/min. The bare Au sensor chip was obtained from Biosensing Instrument Inc. Instrument operations and data processing were performed by the BI-SPR 2000 control software (version 2.2.0.). Thoroughly degassed 0.01 M PBS (pH 7.4) buffer was used as the carrier solution. Transmission electron microscope (TEM) was carried out on JSM-6380 (JEOL). The inductively coupled plasma atomic emission spectrometry (ICP) was carried out on optima 8300 (Perkinelmer, USA). The UV–visible spectrum was recorded on UV-2450 ultraviolet–visible spectrophotometer (Shimadu, Japan). The FI-IR spectrum was recorded on Spectrum 65 spectrometer (Perkinelmer, USA).

2.3. The synthesis and biofunctionalization of carboxyl group modified Fe_3O_4 MNPs

The carboxyl group modified Fe_3O_4 MNPs (carboxyl-Fe_3O_4 MNPs) was synthesized using a previously reported chemical coprecipitation method (Zhou et al., 2010). In brief, 50 mL aqueous solution containing fulvic acid (2 mmol) was purged with argon to remove oxygen and then heated to reflux (101 °C). Then, 4 mL diluted HCl solution containing 2 mmol $FeCl_3 \cdot 6H_2O$ and 1 mmol $FeSO_4 \cdot 7H_2O$ was injected into the hot solution rapidly, followed by concentrated ammonia solution (25 mL). The mixture was kept refluxing for 2 h and then cooled to room temperature. The products were collected after centrifugation and washed four times with ultrapure water and dispersed in ultrapure water. The water-soluble carboxyl-Fe_3O_4 MNPs was obtained. The original concentration of carboxyl-Fe_3O_4 MNP analyzed by ICP was 7.14 mg/mL, which would be used to prepare other concentrations of carboxyl-Fe_3O_4 MNP solution by dilution and stored at 4 °C.

For optimizing concentration of Ab combined with carboxyl-Fe_3O_4 MNPs, BSA was used as the control sample according to the previous reported method (Zhao et al., 2009). The Ab was conjugated