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A non-competitive surface plasmon resonance immunosensor for rapid detection of triazophos residue in environmental and agricultural samples

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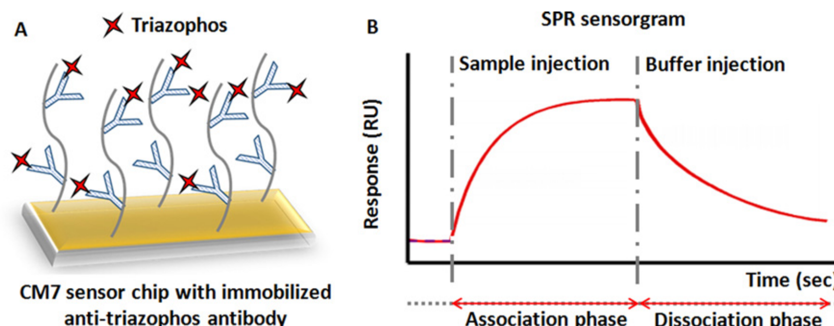
HIGHLIGHTS

- Kinetic analysis of the interaction between triazophos and its monoclonal antibody
- Non-competitive direct SPR immunosensing for triazophos in trace level
- The biosensor method was rapid, reliable and reproducible
- The immunoassay was successfully applied to real samples

GRAPHICAL ABSTRACT



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ABSTRACT

The wide application of an organophosphate pesticide triazophos raises concern on the environmental pollution and the potential risk to human health. Thus, it is crucial to regularly monitor triazophos residue in the environment and agro-products. Herein we described a non-competitive immunoassay for trace detection of triazophos using a direct surface plasmon resonance (SPR) biosensor. Two anti-triazophos monoclonal antibodies (mAbs) were immobilized on the sensor chip and characterized by SPR-based kinetic analysis. The mAb with relatively slow dissociation rate was used for direct immunosensing of triazophos. The biosensor assay showed a high specificity and a low detection limit of 0.096 ng mL^{-1} to triazophos, with the linear detection range of 0.98–8.29 ng mL^{-1}. Under the optimal condition, the sensor chip could be regenerated for 160 cycles at least. Moreover, the sensitive method was applied to determine triazophos in the spiked environmental water and agricultural products, as well as in unknown real-life samples (including Chinese cabbage, cucumber, and apple). Desirable results demonstrated that the newly-developed immunosensor could be used as a rapid, convenient, and reliable tool to regularly monitor triazophos and meet the detection requirement of its maximum residue limits.

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1. Introduction

There is a growing concern on the widespread use of pesticides and their possible impacts on public health. Triazophos (*O*, *O*-diethyl-*O*-(1-phenyl-1H-1, 2, 4-triazol-3-yl) phosphorothioate), an alternative to

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highly-toxic organophosphate pesticides, has been extensively used in agriculture practices to control insect pests on cereals, vegetables, fruits, tea, and so on. For example, in China, the annual usage of triazophos was around 10,000 ton in average during the last 5 years. Due to its high chemical and photochemical stability (http://sitem.herts.ac.uk/aeru/iupac/Reports/653.htm), triazophos may remain in the natural environment, enter aquatic ecosystems, and lead to side effects on aquatic biota such as fish (Zhu et al., 2014; Liu et al., 2015a). Recent researches also manifested that triazophos induced oxidative stress and histomorphological changes in rats (Sharma and Sangha, 2014; Sharma et al., 2015; Jain et al., 2011), thereby having potential risk to human health. Maximum residue limits (MRLs) for triazophos in different food products have been announced by many countries, generally ranging from 0.01 to 0.2 mg kg^{-1} (see the details in the Supplementary material). Thus, it is essential to regularly monitor triazophos residue in the environment and agricultural products.

Classic analytical techniques such as high performance liquid chromatography and gas chromatography coupled with different detection modes were maturely used to determine triazophos residue in various samples (Fu et al., 2009; Zhao et al., 2014; Andrade et al., 2015; Hayward et al., 2015). These methods are accurate and reliable, but require skilled technicians, sophisticated instrumentation, high consumption of organic solvent, and complicated sample pretreatment. Acetylcholinesterase-based biosensors for triazophos (Ju et al., 2015; Du et al., 2007) are rapid, easy-to-use, but lack high specificity, as the acetylcholinesterase can be inhibited by both organophosphate and carbamate pesticides. As a popular screening methodology, immunodiagnostics of different formats, such as enzyme-linked immunosorbent assay (ELISA) (Liang et al., 2007; Gui et al., 2010; Gui et al., 2006; Jin et al., 2008), microbead-based immunoassay (Du et al., 2015; Liang et al., 2013; Guo et al., 2013), chemiluminescent enzyme immunoassay (CLEIA) (Jin et al., 2012; Chen et al., 2015), gold immunochromatographic strip test (Guo et al., 2009; Gui et al., 2008), and fluorescence polarization immunoassay (Liu et al., 2016a) have been successfully developed for rapid detection of triazophos residue in food and environmental samples, with the detection limits lower than 0.01 mg L^{-1}. However, those immunoassays are based on the competitive indirect detection mode and the reaction signal is inversely proportional to the amount of analyte, which differs from the direct sandwich mode for large molecules and may be misjudged by inexperienced people. A method that can directly determine the target itself (instead of the unbound antibody from an inhibition reaction) will be more attractive in terms of accelerated procedure, reduced use of bioreagents, and improved confidence of the assay. Till now, only a noncompetitive rapid piezoelectric immunosensor for direct determination of triazophos was reported (Huang et al., 2010), but the detection limit (0.04 mg L^{-1}) and working range (0.1–100 mg L^{-1}) could hardly reach the MRL requirement. Other burgeoning immunosensors that have potential for direct measurement were not exploited for trace detection of triazophos.

Surface plasmon resonance (SPR) technology is an optical detection platform that offers real-time and label-free analysis of molecular interaction. In the past two decades, SPR-based immunosensors have been widely applied to detect large molecules, where the analyte's high mass and the use of sandwich immunoassay format can lead to high signal and thus desirable sensitivity (Scarano et al., 2010; Riedel et al., 2014). In contrast, low-molecular-weight (LMW, <1 kDa) compounds present in trace level are very difficult to be directly determined by traditional SPR immunosensors, owing to the small change in the refractive index induced by the binding of those analytes on the sensor surface (Gouzy et al., 2009). Therefore, competitive inhibition SPR immunoassays were often developed for trace analysis of pesticides. That is, a pesticide-protein conjugate served as the competitor was immobilized on the sensor chip and the solution competition occurred when injecting the mixture of antibody and analyte (Hirakawa et al., 2015a; Mauriz et al., 2012; Estevez et al., 2012; Hirakawa et al., 2015b); otherwise, the antibody was immobilized on the sensor chip and the surface competition happened when injecting the mixture of competitor and analyte (Mitchell, 2010). In recent years, other strategies to improve the sensitivity of direct SPR non-competitive immunoassay for pesticides have been explored by employing nanoparticle lables as signal enhancement reagents (Liu et al., 2014; Liu et al., 2015b). Although these methods can meet the requirement for trace detection of pesticides, they need longer assay time, more reagents and complex labeling process. Still, direct SPR non-competitive immunosensors providing more rapid and straightforward diagnostics deserve further investigations for detection of small molecules, despite more challenges.

Recent improvements in SPR instrumentation including lower noise valves and advanced microfluidics with stronger vacuum pumps, have decreased the total noise in these systems and hence resulted in better sensitivity and reliability. However, attempts for trace analysis of LMW analytes using direct SPR non-competitive immunoassays were limited to very few targets (Yakes et al., 2014; Munoz et al., 2011). In this work, we first characterized two monoclonal antibodies (mAbs) against triazophos by ELISA and SPR-based kinetic analysis. After comparison, the optimal mAb was used to develop a non-competitive SPR immunosensor for direct monitoring of triazophos residue in environmental and agricultural samples. The key factors in the assay design and the effects of sample matrices on the assay performance were also discussed.

2. Materials and methods

2.1. Reagents and instrumentation

Triazophos, parathion, chlorpyrifos and other related pesticide standards were obtained from Agro-Environmental Protection Institute, Ministry of Agriculture (Tianjin, China). Each pesticide stock solution with a concentration of 1 mg mL^{-1} was prepared in dimethylsulfoxide (DMSO) and stored at 4 °C. Sensor chip (CM7), phosphate buffered saline (PBS, 0.01 M, pH 7.4) containing 0.05% (v/v) polysorbate surfactant P20 (PBS-P+), the amine coupling kit containing 0.1 M *N*-hydroxysuccinimide (NHS), 0.4 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 1 M ethanolamine-HCl (pH 8.5), 10 mM sodium acetate (pH 4.5, 5.0, and 5.5), immobilization and regeneration buffers were procured from GE Healthcare Bioscience AB (Upsala, Sweden). All buffer solutions were prepared with ultra-pure water acquired from a Milli-Q purification system (Millipore, Bedford, USA) and were passed through the membrane filter (pore size, 0.22 μm, Millipore) prior to use. All SPR tests were carried out at 25 °C using Biacore T200 biosensor system (GE Healthcare, Madison, USA). The data acquisition software was Biacore T200 Evaluation Software Version 3.0.

2.2. Production of mAbs against triazophos

Two kinds of mAbs specific to triazophos were previously developed in our laboratory, named as 4B2 (Jin et al., 2008) and 8C10 (Liu et al., 2016b). The mAbs were purified and dissolved in 10 mM phosphate-buffered saline (PBS) to a final concentration of 1 mg mL^{-1}. To make sure the protein activity, titers of mAbs were measured by non-competitive indirect ELISA (iELISA) and the sensitivity to triazophos (IC_{50}, 50% inhibitory concentration) was determined by competitive iELISA. The conjugate of triazophos hapten-ovalbumin (THBu-OVA) (Gui et al., 2006) was used as the coating detective antigen. Standard competition curves were acquired by plotting the inhibition rate against the logarithm of analyte concentration. The ELISA procedures were described in our previous work (Liu et al., 2016b).

2.3. Sensor immunochip preparation

A sensor chip CM7 coated with a high density of carboxymethylated dextran was put into Biacore T200 system. Initially, a solution of 70% (w/

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