Analytical and Bioanalytical Chemistry
https://doi.org/10.1007/s00216-019-01990-0

RESEARCH PAPER

Oriented assembly of surface plasmon resonance biosensor through staphylococcal protein A for the chlorpyrifos detection

Qian Li[1] · Xiaowen Dou[1] · Lei Zhang[1] · Xiangsheng Zhao[2] · Jiaoyang Luo[1] · Meihua Yang[1,2]

Received: 14 March 2019 / Revised: 2 June 2019 / Accepted: 18 June 2019

Abstract

In this study, we report a direct surface plasmon resonance (SPR) biosensor based on an oriented assembly of antibody for the rapid detection of chlorpyrifos residue in agricultural samples. In this covalent-orientated strategy, staphylococcal protein A (SPA) was first covalently bound to the surface for monitoring chlorpyrifos residue, with subsequent binding of the antibody in an orientated fashion via its fragment crystallizable (Fc) region. Consequently, the SPA-modified biosensor exhibited a satisfactory specificity and a low detection limit of 0.056 ng mL^{-1} for chlorpyrifos, with a linear detection range of 0.25–50.0 ng mL^{-1}. Under optimal conditions, the sensor chip could be regenerated for at least 210 cycles. The results presented here indicate that the SPA-modified sensor chip can successfully improve the sensitivity and obviating the need of the modification of the antibody. The developed SPR biosensor method has the great potential for rapid, sensitive, and specific detection with broad applications in areas of environmental monitoring and food safety.

Keywords SPR biosensor · Antibody immobilization strategy · Chlorpyrifos · Staphylococcal protein A (SPA) · Agricultural product monitoring

Introduction

Chlorpyrifos (O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl)-phosphorothioate, MW 350.59) is one of the most frequently used broad-spectrum organophosphate insecticides to control harmful insects and enhance production of crops. However, incorrect and uncontrolled use has resulted in increasing public concern surrounding food and environment safety. Chlorpyrifos appears as one of the most detected pesticides agriculturally [1]. There is evidence that the toxicity of pesticide residues found in food samples could potentially result in a risk to human health [2–4]. Many countries have announced maximum residue limits (MRLs) for chlorpyrifos in different food products, which generally range from 0.01 to 1.0 mg kg^{-1}. Therefore, for the benefit of public health, the development of a rapid, highly sensitive, and specific method that can be used to regularly monitor chlorpyrifos residue in agricultural products is of the utmost importance and urgency.

Several sensitive bio-analytical techniques based on immunologic concepts were developed as rapid and cost-effective tools for detecting trace amounts of pesticides such as enzyme-linked immunosorbent assays (ELISA) [5], fluorometric competitive immunoassays [6], gold immunochromatographic strip tests [7], fluorescence polarization immunoassays [8], and electrochemical immunoassays [9]. However, these methods require many preparation steps with biological reagents, long incubation periods in order to immobilize enzymes or other biomarkers, and repeated washing steps, all of which make automation for regular analysis of samples difficult. Therefore, the development of a simple and real-time determination assay for ensuring food quality is desirable.

In order to resolve the aforementioned issues, a more effective method is needed that could be applied to a broad range of food products. In this regard, SPR biosensor provides an excellent alternative to traditional analytical techniques. SPR is

✉ Meihua Yang
yangmeihua15@hotmail.com

[1] Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, Institute of Medicinal Plant Development, Chinese Academy of Medical Science, Peking Union Medical College, 151 Malianwa North Road, Beijing 100193, China

[2] Hainan Branch of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Yaogu 4 Road, Xiuying District, Haikou 570311, Hainan, China

Published online: 05 July 2019

an important optical physical phenomenon based on the excitation of surface plasmons, where SPR sensors monitor changes in the refractive index of the biomolecular interactions on metal surfaces [10–12]. The advantages of SPR sensors include a rapid response time, the ability for real-time monitoring, being less time consuming, high efficiency, and not requiring labelling techniques [12, 13]. These beneficial properties have contributed to remarkable growth in the use of the SPR biosensor in numerous analytical fields, medical diagnostics [14, 15], environmental monitoring [16], and food quality and safety analysis [17, 18]. For sensitive pesticide analysis, the indirect competitive immunoassay has attracted great attention from researchers because it is more advantageous than the direct method for the detection of low-molecular-weight compounds [19]. However, the direct assay has some advantages, mainly that it has accelerated steps, saves reagent, and simplifies the experimental process. In recent years, many strategies using various types of nanoparticles as signal enhancement material have been explored in order to improve the sensitivity of direct SPR immunoassay for pesticides [20–22]. Although the detection based on nanomaterials are developed, it still faces the problems of homogeneous and controllable labeling techniques (the key factor for reproducibility and accuracy of results), the stability in food detection as well as the repeatability of nanomaterials. Therefore, SPR biosensors with no need of competitive immune reaction and complicated nanomaterials preparation is in high demand to fulfill a direct readout of the small pollutants level, like chlorpyrifos.

In this study, a novel strategy for the rapid on-line detection of chlorpyrifos was developed based on the direct format using an SPR biosensor. The biosensor was modified with staphylococcal protein A (SPA) as a supported protein layer on the SPR chip. Due to the high degree of protein orientation of the SPA on the sensor surface, this biosensor can capture antibody binding specifically to the Fc region without interacting at the antigen binding sites [23, 24]. In a non-competitive immunoassay, the analyte binds directly to an antibody that has been previously immobilized on the immunochip surface. Because the SPR signal is directly proportional to the concentration of the analyte in the sample solution, the non-competitive immunoassay provides the most simple and rapid method of detection. After comparing these assays, we also examined the key factors involved in the assay design as well as the effects of sample matrices on the assay performance. This strategy has many meaningful advantages in that it is rapid, on-line, highly sensitive, and does not require complicated preparation, all of which lend it great potential for the efficacious detection of small analytes at trace levels in a wide range of fields for the monitoring of food safety.

Materials and methods

Reagent and materials

The common reagents mercaptoundecanoic acid, epichlorohydrin, bromoacetic acid, glycine, bovine serum albumin (BSA), 2-(N-morpholino)ethanesulfonic acid hydrate, 1-ethyl-3-(3-dimethylamino-prophl) carbodiimide hydrochloride (EDC) and n-hydroxysuccinimide (NHS), and inorganic salts for buffer preparation (i.e., PBS (phosphate-buffered saline)) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from the Tianjin Chemical Reagent Factory. Tween 20 and methanol were purchased from Quantum and Merck, respectively. All other chemicals and reagents were purchased from Aladdin Industrial Corporation (Shanghai, China).

Chlorpyrifos-BSA (1.0 mg mL^{-1}) and chlorpyrifos monoclonal antibody (1.0 mg mL^{-1}) were purchased from Wuxi Determine Bio-Tech Co., Ltd. SPA was purchased from Beijing Biosynthesis Biotechnology Company.

Chlorpyrifos and other related pesticide standards were obtained from the Agro-Environmental Protection Institute, Ministry of Agriculture (Tianjin, China). All of the working solutions of the analytical standards were prepared in methanol.

All reagents were of analytical grade and ultra-pure water was used in all experiments. Chlorpyrifos-mAb was dissolved with 0.01 mol L^{-1} PBS (pH 7.4). Chlorpyrifos-BSA was dissolved with 0.01 mol L^{-1} sodium acetate buffer solution (pH 4.5). The blocking buffer was 1 mol L^{-1} methanolamine (pH 8.5).

Apparatus

SPR measurements were obtained on an SPR instrument (Beijing Inter-bio Tech CO, T400) using the Kretschmann configuration. Changes in the angle of the reflected light indicate shifts in mass on the surface and were detected with a charge-coupled device. Thoroughly degassed 0.01 mol L^{-1} PBS (pH 7.4) buffer was used as the carrier solution and was applied at a constant speed of 10 μL min^{-1}.

Oriented assembly of SPR biosensor based on staphylococcal protein A

The CM5 sensor chip with carboxymethylated dextran matrices on gold surfaces was purchased from Inter-bio (Beijing, China). In the preliminary test, the sensor chip was rinsed with 0.01 mol L^{-1} PBS (pH 7.4) at a flow rate of 10 μL min^{-1} for 10 min. The mixed solution of EDC and NHS (0.4 mol L^{-1} and 0.1 mol L^{-1}) in 0.1 mol L^{-1} 2-(N-morpholino) ethanesulfonic acid buffer (MES, pH 6.0) was then injected into the instrument to activate the carboxylic groups on the

chip for 15 min. Next, 1.0 mg mL^{-1} SPA was diluted by 0.01 mol L^{-1} PBS (pH 7.4) to 200 μg mL^{-1}, and it was covalently immobilized onto the surface of the chip using the active ester method. After 1 h, the free carboxyl groups on the surface were blocked with 1 .0 mol L^{-1} methanolamine (pH 8.5) to deactivate any unreacted sites for 10 min. Then 50 μg mL^{-1} chlorpyrifos-mAb was injected into the channel to bind with the Fc region of the SPA. This procedure was sustained 15 min, after which the antibody was immobilized on the sensor surface and subjected to the binding interaction with the analyte of interest.

Establishment of calibration curves immunoassay detection format

Chlorpyrifos measurements were performed in triplicate using a direct immunoassay. The chlorpyrifos standard solutions were diluted with 0.01 mol L^{-1} PBS containing 5% methanol for analysis and used to generate the calibration curve at the final concentrations of 0.005 to 500 ng mL^{-1}, with zero concentrations used as the blank control. The sample was then flowed over the sensor surface at a rate of 10 μL min^{-1} for 10 min in order to recognize chlorpyrifos-mAb on the SPA layer of the chip's surface. For repeated use, the sensor chip was flowed by regeneration solution (10 mmol L^{-1} glycine-HCl, pH 3.0) for 5 min to dissociate the antibody from the surface of chip. The corrected and blank-deducted response was plotted against the chlorpyrifos concentration to yield a standard sigmoidal curve for the SPR immunoassay.

Cross-reaction test of the biosensor

The ability to evaluate the specificity of the assay for selectively detecting the target analytes is an important capability of an SPR biosensor. The selectivity of this biosensor was interrogated through the testing of a series of structural and functional analogues of chlorpyrifos, including chlorfenvinphos, bromophos-ethyl, dichlofenthion, and chlorpyrifos-methyl. The concentrations of all the selected pesticides were 1.0 mg mL^{-1}. These selected pesticides were individually diluted with 0.01 mol L^{-1} PBS at the same concentration of 25 ng mL^{-1} containing 5% methanol.

Spiked recovery test

Agricultural products such as maize, cabbage, apple, and medlar were purchased from the supermarket (Beijing, China). The smashed samples (4 g of each) were extracted with 10 mL of 80% (*v*/*v*) methanol for 30 min using an automatic shaker. The supernatant was then diluted 10-fold time with 0.01 mol L^{-1} PBS containing 5% methanol for analysis. For all experiments, samples were filtered using 0.45-μm NYLON microporous syringe filter. All of the samples were first confirmed to be chlorpyrifos-free by UPLC-MS/MS and were used in the SPR tests for the matrix effect and spiked recovery determination. For recovery tests, three standard concentrations in the linear range were spiked into blank samples. To validate the SPR sensor performance, samples and recoveries were analyzed using both the SPR sensor and the UPLC-MS/MS method. The UPLC-MS/MS method was carried out using a Waters Acquity™ UPLC-MS/MS system (Xevo G2-XS, Waters, USA). The chromatographic separation was performed on an Acquity BEH C18 column (50 × 2.1 mm, 1.7 μm) from Waters (MA, USA) at a flow rate of 0.30 mL min^{-1}. The mobile phase consisted of acetonitrile (A) and aqueous solution containing 0.1% formic acid (B). The injection volume was 2.0 μL. MS detection was conducted with electrospray ionization (ESI) source in positive mode. Multiple reaction monitoring (MRM) mode was adopted for quantitation.

Results and discussion

Preparation of protein A layer formation sensing platform

One of the key elements for a successful immunoassay is the construction of an efficient recognition surface that can interact quickly and reliably with its associated binding partners or analytes. It is important to create a sensor surface that possesses a specific combination of characteristics with minimum background interference in order to improve its ability for screening various analysis matrices that are complex [19]. An excellent immobilization layer is required for highly oriented coupling to antibody molecules and offers enough space in terms of the molecular recognition process. A randomly fixed antibody molecule may mask its Fab fragment because of steric hindrance and would thus partially lose its ability to bind antigen [24]. As one kind of Fc fragment receptor, directional proteins exhibit certain geometric orientation advantages, which can expose the Fab fragment of the antibody outside the modified membrane without interfering with the immunoreactivity of the antibody and antigen. To some extent, this directional immobilization mechanism has little effect on the immune activity of antibodies, so the binding ability between antigen and directional fixed antibody is 2 to 8 times higher than that of random fixed antibody, which greatly improves the effectiveness of antibody recognition [24]. A high degree of orientation for the capture antibody on a sensor surface can be attained with a sub-layer of proteins, such as protein A. A cell wall component of *Staphylococcus aureus*, protein A binds with the Fc region of an antibody, without interacting at the antigen binding sites [5]. SPA controlled antibody immobilization results in highly efficient immunoreactions and enhanced binding capacity to antibody

therefore improving the sensitivity of detection performance. In this work, we fabricated an SPR biosensor for direct detection of chlorpyrifos by immobilizing the chlorpyrifos-mAb to a sensor chip using directional immobilization with the SPA layer. We then directly monitored the interaction between chlorpyrifos in the solution and the chlorpyrifos-mAb located on the chip surface (Fig. 1).

The development of the SPA-functionalized sensor chips used for this study's strategy of orientated immobilization of antibody was optimized for SPA concentration (Fig. 2). In order to achieve the optimal performance from the SPA sensing layer, we evaluated four concentrations of SPA (50.0, 100.0, 200.0, and 400.0 μg mL^{-1}) and five concentrations of antibody (1.0, 10.0, 20.0, 50.0, and 100.0 μg mL^{-1}) with the sensor system. Each concentration of SPA was separately immobilized on the chip surface for 10 min, and then the antibody was injected into the SPR cell at 10 μL min^{-1} to bind with SPA layer for 10 min.

Figure 2a indicates that there was a stronger signal with higher amounts of SPA binding on the surface. However, the response signals resulting from 200 and 400 μg mL^{-1} SPA were maintained at 450 RU, which was not proportional to the SPA concentration. It was supposed that excessive SPA could not be completely combined on the chip effectively due to the limited carboxyl sites on the chip surface.

Figure 2b indicates that, when the SPA concentration was 200 μg mL^{-1}, the binding signals increased along with the amount of antibody. Yet, the higher antibody concentration could not cause the obvious change in response signal. When the antibody concentration was 50.0 μg mL^{-1}, the strong binding signal was maintained at 360 RU. Thus, the 50.0 μg mL^{-1} antibody concentration was determined to be appropriate for coupling with the SPA layer.

In order to further confirm the superiority of this operating strategy and the binding capacity of the chip surface, we employed the most widely used strategy, a direct immunoassay, at the same concentration of antibody conjugation on a traditional carboxymethylated dextran-sensing layer. Figure 2b, c demonstrate that at the same concentration of antibody, maximum binding signal was maintained at 160 RU based on a traditional carboxymethylated dextran-sensing layer, which was far below 360 RU based on SPA layer. The stronger signals were obtained from the chip modified with SPA using the oriented immobilization of antibody strategy.

In consideration of the existence of steric hindrance and possibility that the randomly fixed antibody molecule might mask its Fab fragment and lose its ability to bind antigen partially, this experimental result demonstrated that the directional fixation mechanism was a favorable strategy to offer a suitable layer for further orientated immobilization of the antibody.

Assay sensitivity

To assess the assay sensitivity using direct assays based on the SPA layer, we established calibration curves by detecting a series of chlorpyrifos solutions ranging from 0.005 to 500 ng mL^{-1}. The corrected and blank-deducted signals at the report point were recorded and plotted against the chlorpyrifos concentration. These solutions (150 μL) were individually flowed on the biosensor surface and measurements were

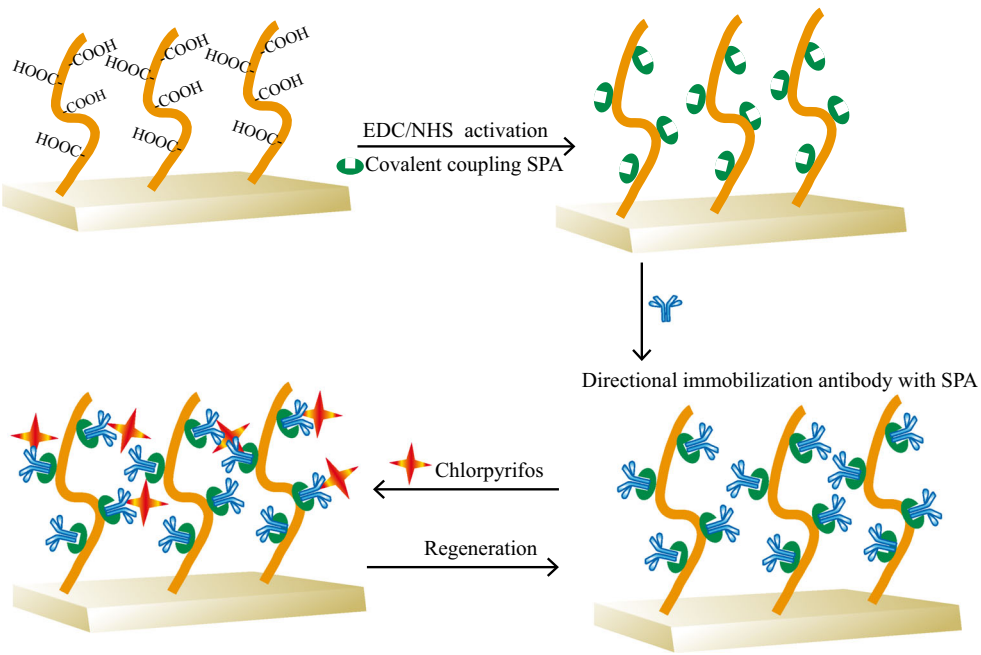

Fig. 1 A schematic diagram of the experimental procedure

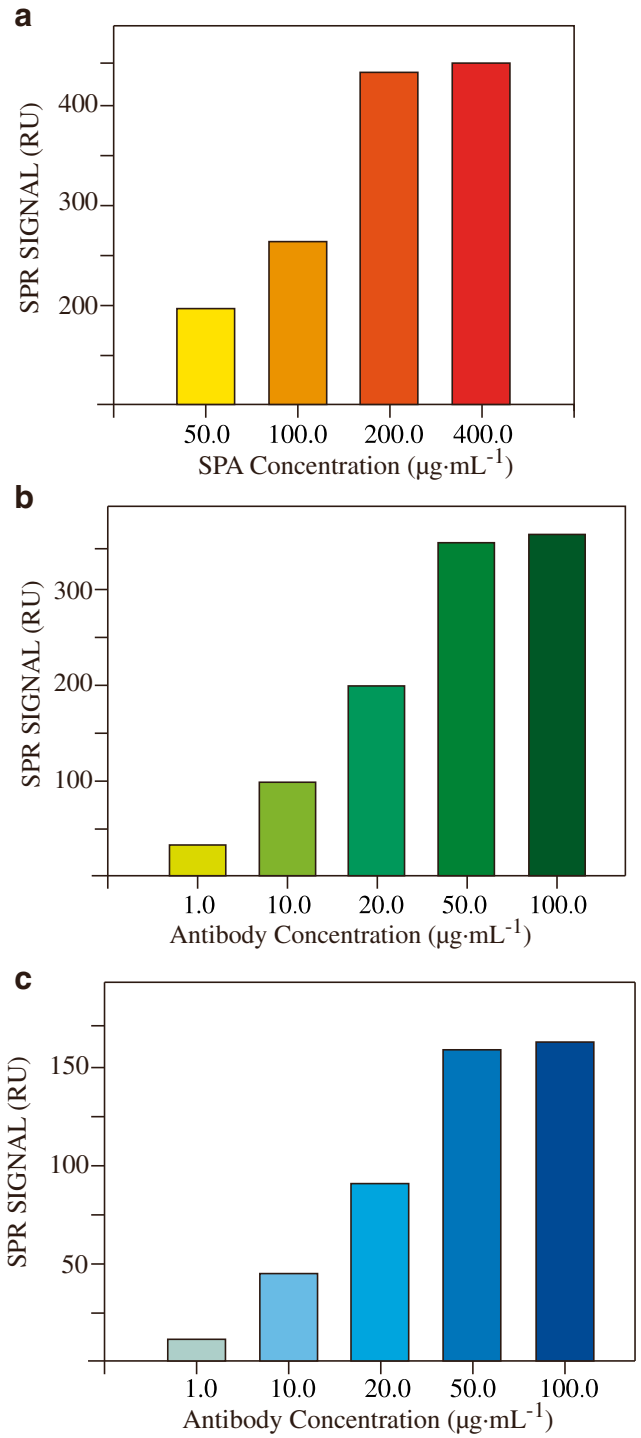

Fig. 2 **a** The SPR signals were induced by different concentration of SPA binding on the chip surface. **b** The SPR signals were induced by the different concentration of antibody binding on the chip surface modified with SPA. **c** The SPR signals were induced by the different concentration of antibody binding on the CM5 chip surface ($n = 3$)

obtained in a similar manner using the direct method to monitor the signal without the SPA layer.

Figure 3a displays an average calibration curve of chlorpyrifos detected using an SPA layer biosensor. Using the fitted four-parameter logistic equation, we observed that a good linear relationship was obtained with chlorpyrifos concentrations ranging from 0.25 to 50 ng mL^{-1}. The regression equation was $y = 47.56 + 31.62x$ ($R^2 = 0.998$) and the limit of detection (LOD) was 0.056 ng mL^{-1}, using $S/N = 3$ where the noise level is the standard deviation of 6 replicate measurements at a concentration of zero. The error bars for the data points indicated good repeatability for the assay. Figure 3b shows an average calibration curve of chlorpyrifos detected without the SPA layer with chlorpyrifos concentrations ranging from 5.0 to 25 ng mL^{-1}. The regression equation was $y = 12.45 + 28.25x$ ($R^2 = 0.997$) and the LOD was 0.73 ng mL^{-1}. This experimental result demonstrated that the oriented immobilization of antibody strategy was the more favorable strategy to offer a suitable layer for further detection of small molecular compounds. The limit of determination of the present biosensor is 0.056 ng mL^{-1}, which was lower than the traditional method, and this result was also lower than others reported by literatures [25, 26], 55 ng mL^{-1} and 7.5 nM.

Immunochip stability

Method characteristics of stability need to be verified to ensure measurement repeatability and accuracy. The stability assay showed that the SPA layer was fairly tolerant to the regeneration conditions (10 mmol mL^{-1} glycine-HCl, pH 3.0) that break the binding between the antibody and analyte, maintaining a sensor response signal around 10 RU. As shown in Fig. 4, the baseline of the SPR signal remained stable around 10 RU until 125 regeneration cycles, and within 5 days, the sensor immobilization signal of chlorpyrifos at 25 ng mL^{-1} was stable around 95 RU until 150 regeneration cycles. After that, the immunochip began to deactivate and the SPR response signal weakened. The baseline of the SPR signal remained around 18 RU, and the sensor response signal maintaining around 83 RU. At 210 regeneration cycles, the immune layer was destroyed and the antibody was not able to completely recognize chlorpyrifos. Within a period of 7 days, the SPR baseline signal maintaining around 16 RU, and the immobilized chip would maintain effectiveness for 210 regeneration cycles.

Assay selectivity

The specificity of the assay to selectively detect target analytes is an important capability of an SPR biosensor. Here, we investigated the specificity of the biosensor by performing cross-reactivity studies to a series of structural and functional analogues of chlorpyrifos, including chlorfenvinphos, bromophos-ethyl, dichlofenthion, and chlorpyrifos-methyl. We dissolved these analogues in 0.1 mol mL^{-1} PBS containing 5% methanol to create solutions with concentrations of 25 ng mL^{-1} and then tested them using the same direct assay as for chlorpyrifos. As shown in Fig. 5, other four tested pesticides had negligible interference on the detection of targets,

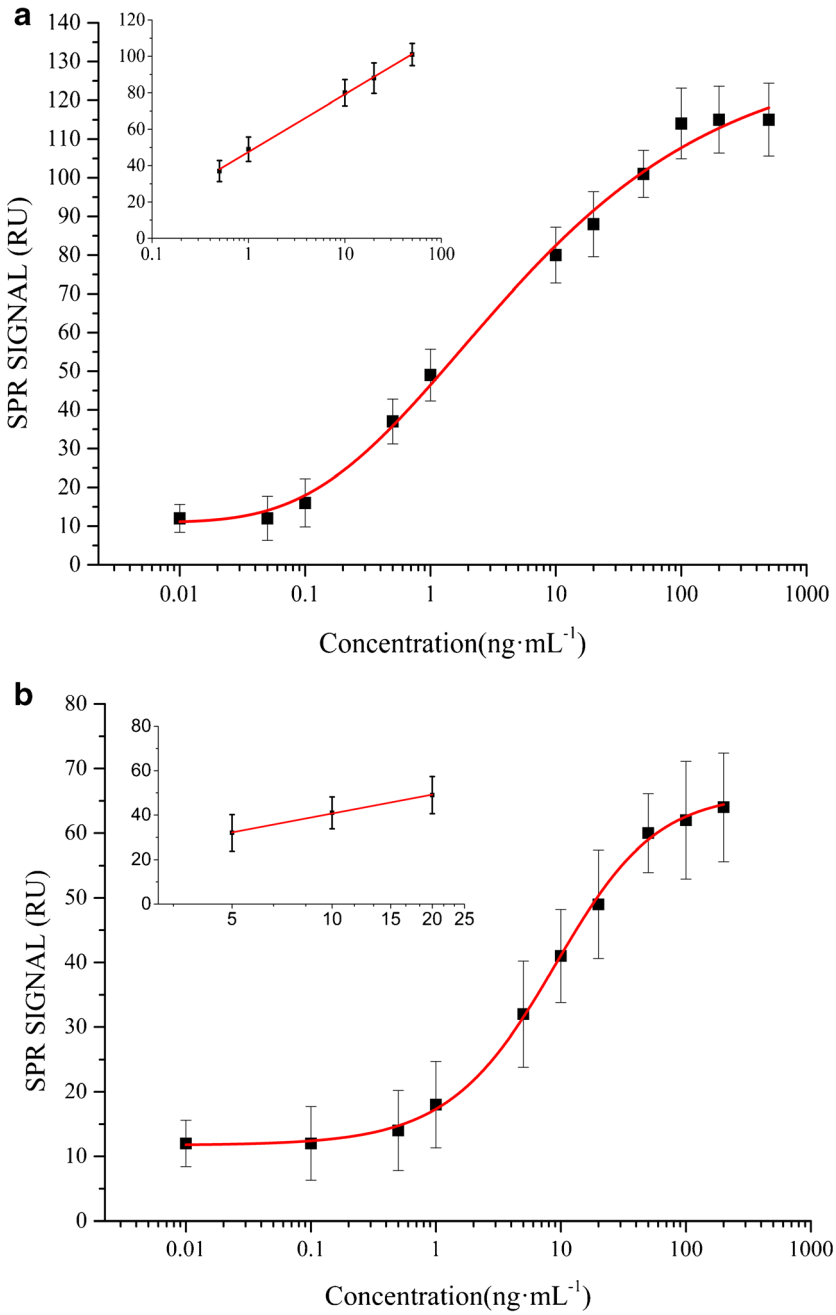

Fig. 3 The relationship between the SPR signal and the concentrations of chlorpyrifos. The biosensor based on SPA layer (**a**) and the biosensor based on traditional carboxymethylated dextran-sensing layer without SPA (**b**)

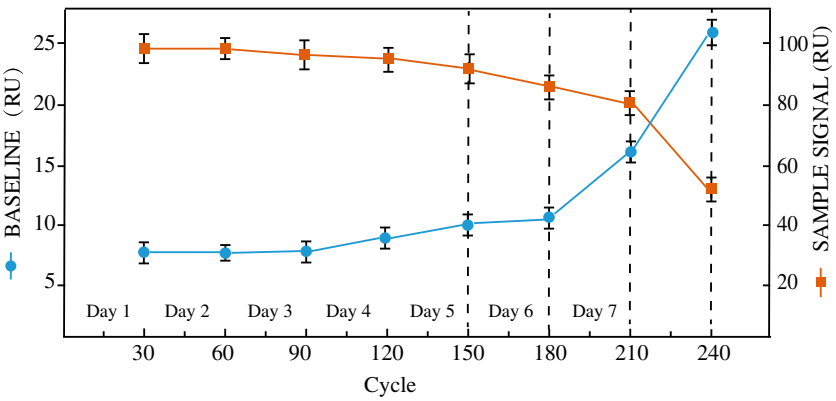

Fig. 4 Stability of the SPA-modified biosensor by repeatedly recording the SPR response of chlorpyrifos (25.0 ng mL^{-1}) at interval of 30 cycles per day and sample response after using the regeneration solutions (data are the mean values of 3 replicates)

suggesting that the newly designed biosensor possessed excellent selectivity for chlorpyrifos over other pesticides.

Matrix interference

Because different sample matrices can cause different types of interference with the chip surface, separate calibration curves should be set up for each sample matrix. However, this practice is not practical to implement for the detection of various kinds of samples by rapid methods such as SPR immunoassays. Therefore, this experiment selected several representative agricultural products (maize, cabbage, apple, and medlar) to investigate the effects of matrix interference.

In the preliminary assay, we assessed matrix interference by comparing a matrix-free standard curve with calibration curves obtained in the agricultural product matrices at different dilution times. At first, the agricultural products were rapidly extracted with 80% (*v*/*v*) methanol without purification, but we found that the extraction solution seriously interfered with the immunochip's capability for detection of chlorpyrifos. The matrix interference could be reduced through the generous dilution of samples, but equally important to consider is that the pesticide residue levels in the actual samples were very low. So it is extremely necessary to choose the appropriate dilution time to remain within the dynamic range of the assay and avoid the possibility of obtaining chlorpyrifos in real samples at levels that would be undetectable. When the curves of the 10-fold dilutions were very close to the standard curves made from matrix-free buffer solutions. Comparing the matrix interference of different samples, we found that the cabbage and apple exhibited a lower interference effect than maize. Through further comparative analysis of matrix interference with two different layers on the chip surface, much greater dilutions (50-fold for maize, and 20-fold for cabbage, apple, and medlar) was needed in order for the sample extract to minimize the matrix effects on the SPR tests without the SPA layer. This result corroborated the concept for the use of the oriented immobilization of antibodies, leading to improved effectiveness of antibody recognition and reduced matrix interference when samples were injected onto the surface of the immunochip.

Recovery tests

In order to confirm the applicability of the SPR sensor in determining chlorpyrifos residues in agricultural products, we carried out recovery examination. The blank samples were analyzed with UPLC-MS/MS to confirm that chlorpyrifos was undetectable.

After simple pretreatment, we detected the chlorpyrifos-spiked samples at final concentrations of 1.0, 25.0, and 50.0 ng mL^{-1} using the SPR sensor.

Table 1 lists the recoveries of chlorpyrifos in the spiked samples analyzed by the SPR biosensor. For the spiked agricultural samples, a simple dilution with the running buffer was useful for eliminating the matrix influence, and the mean recoveries of spiked samples ranged from 86.9 to 119.2%. Coefficients of variation were all below 11.2%, indicating an acceptable precision. The experimental results demonstrated that the developed SPR immunoassay had good accuracy and would generally meet the detection requirement of chlorpyrifos MRLs in agricultural

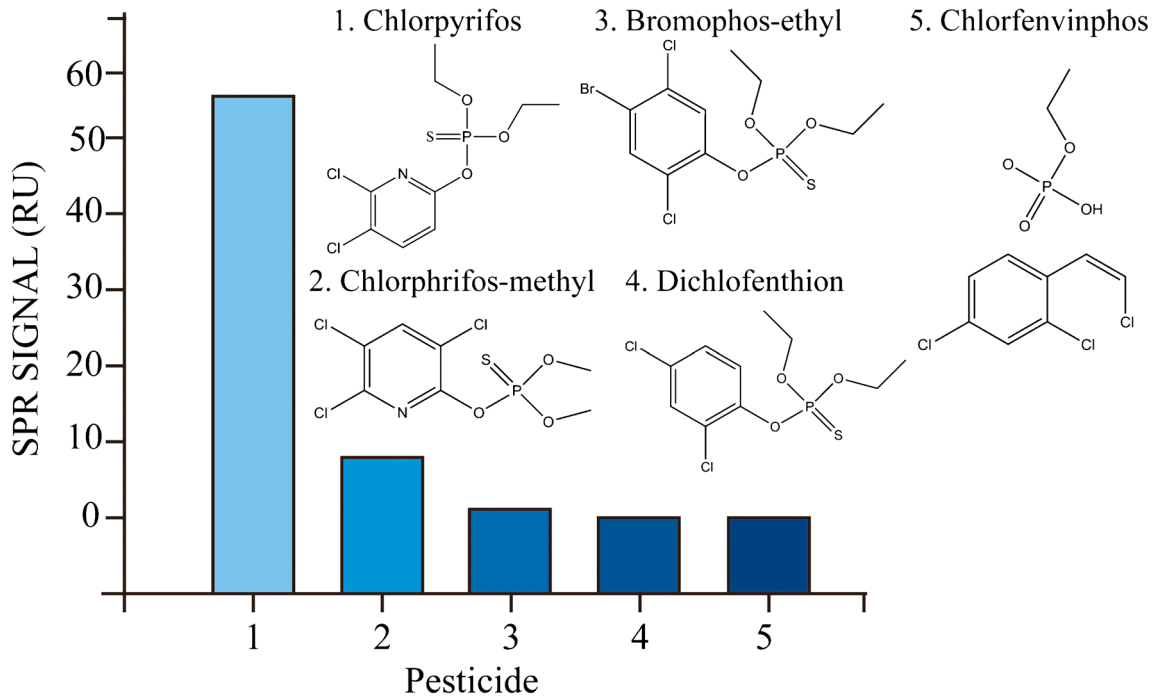

Fig. 5 Selectivity of the SPA-modified biosensor towards different pesticides at each concentration of 25 ng mL^{-1} ($n = 3$)

Table 1 Recovery results of chlorpyrifos in spiked samples using the SPR biosensor *(n = 3)*

Sample	Spiked × 10^{-3} (mg kg^{-1})	Detected × 10^{-3} (mg kg^{-1})	Mean recovery (%)	RSD (%)
Maize	2.5	2.5 ± 0.48	119.2	11.2
	62.5	62.5 ± 4.36	106.9	7.8
	125.0	125.0 ± 11.52	109.2	6.4
Cabbage	2.5	2.5 ± 0.21	91.6	9.4
	62.5	62.5 ± 4.28	106.8	8.8
	125.0	125.0 ± 8.64	93.1	7.1
Apple	2.5	2.5 ± 0.35	86.9	10.8
	62.5	62.5 ± 6.37	110.2	9.3
	125.0	125.0 ± 15.77	107.8	7.2
Medlar	2.5	2.5 ± 0.27	110.8	9.8
	62.5	62.5 ± 5.85	90.7	8.4
	125.0	125.0 ± 9.26	92.6	8.1

products, as mentioned above. The recovery results implied that this proposed method was feasible for the detection of chlorpyrifos residue in food samples.

Analysis of unknown real-life samples

Table 2 shows the results of chlorpyrifos residue in some unknown real samples detected with both the UPLC-MS/MS method and the proposed SPR assay. Out of 20 samples, five were found to be chlorpyrifos positive, with levels ranging from 12.3 to 102.8 ng kg^{-1} as detected by the biosensor tests. By comparison, the results from the UPLC-MS/MS were largely consistent with those obtained from the SPR assay, where the positive data ranged from 10.8 to 99.6 ng kg^{-1}. The chlorpyrifos determinations in real samples were validated by chromatographic methods, which yielded results consistent with one another. The good correlation with the UPLC-MS/MS method ensures the SPR assay's capability as a

Table 2 Analysis of chlorpyrifos residues in unknown real-life samples by the SPR assay and UPLC-MS/MS[a]

Sample	No.	Detected by SPR × 10^{-3} (mg kg^{-1})[b]	Detected by UPLC-MS/MS × 10^{-3} (mg kg^{-1})
Maize	1	ND[c]	ND[c]
	2	ND[c]	ND[c]
	3	ND[c]	ND[c]
	4	ND[c]	ND[c]
	5	ND[c]	ND[c]
Cabbage	1	ND[c]	ND[c]
	2	ND[c]	ND[c]
	3	42.5 ± 2.35	41.7 ± 1.88
	4	ND[c]	ND[c]
	5	ND[c]	ND[c]
Apple	1	ND[c]	ND[c]
	2	86.4 ± 7.37	83.4 ± 5.22
	3	102.8 ± 9.25	99.6 ± 8.41
	4	ND[c]	ND[c]
	5	ND[c]	ND[c]
Medlar	1	12.3 ± 1.37	10.8 ± 1.02
	2	ND[c]	ND[c]
	3	ND[c]	ND[c]
	4	37.2 ± 5.85	35.8 ± 4.34
	5	ND[c]	ND[c]

[a] The limits of detection/quantification (LODs/LOQs) by UPLC-MS/MS were 0.5 ng mL^{-1} /1.5 ng mL^{-1}

[b] Detected concentrations were mean ± standard deviation ($n = 3$)

[c] ND (not detectable), less than the corresponding LOD

valuable analytical screening tool for the continuous monitoring of chlorpyrifos.

Conclusion

The work presented herein features for the first time a simple, rapid, and sensitive method for the detection of trace chlorpyrifos using a direct SPR biosensor based on the oriented immobilization of antibody strategy. The biosensor combined the advantages of oriented immobilized protein and conventional immunoassays for realizing fast detection in real sense, thus improving the binding efficiency of the antibody, simplifying the experimental steps, and saving the use of bio-reagents. Without the competition, the pesticide haptens that are used in the traditional immunoassays as competitors were not necessary and therefore avoid the expensive synthesis and the secondary contamination from the experiments.

To summarize, the assay designed was specific and sensitive, with an LOD of 0.056 ng mL^{-1} for chlorpyrifos, and the immunochip could be reused for 210 cycles. This method provides real-time measurements for analysis of samples in only 10 min and requires minimal sample handling, making it less time consuming than conventional techniques currently used in this field. The application of real samples indicated that the newly developed biosensor-based immobilization strategy could be used as a straightforward and reliable tool for the monitoring of chlorpyrifos residue in agricultural products. In addition, this strategy could be extended to the detection of more than chlorpyrifos by simply modifying the sensing surface with a higher number of pesticides. Therefore, the use of direct SPR biosensors is a more rapid and straightforward monitoring method that deserves further investigation for the detection of more small molecule contaminants whenever the device and immunoreagent are available.

Acknowledgments The authors acknowledge Beijing Inter-bio Tech CO., LTD, for providing the surface plasmon resonance device.

Funding information This work was supported by the National Natural Science Foundation of China (No. 81703699, 81573595, and 81603398), the National Project for Standardization of Chinese Materia Medica (No. ZYBZH-Y-JIN-34), CAMS Innovation Fund for Medical Sciences (No. 2017-I2M-1-013), and the Graduate Student Innovation Fund of Peking Union Medical College (No. 2017-1007-14).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Harshit D, Charmy K, Nrupesh P. Organophosphorus pesticides determination by novel HPLC and spectrophotometric method. Food Chem. 2017;230:448–53.
2. Gupta SC, Mishra M, Sharma A, Deepak Balaji TG, Kumar R, Mishra RK, et al. Chlorpyrifos induces apoptosis and DNA damage in Drosophila through generation of reactive oxygen species. Ecotoxicol Environ Saf. 2010;73(6):1415–23.
3. Sotomayor V, Lascano C, D'Angelo AMP, Venturino A. Developmental and polyamine metabolism alterations in Rhinella arenarum embryos exposed to the organophosphate chlorpyrifos. Environ Toxicol Chem. 2012;31:2052–8.
4. Khalil AM. Toxicological effects and oxidative stress responses in freshwater snail, Lanistes carinatus, following exposure to chlorpyrifos. Ecotoxicol Environ Saf. 2015;116:137–42.
5. Lee W, Byung Keun O, Min Bae Y, Paek SH, Hong Lee W, Choi JW. Fabrication of self-assembled protein a monolayer and its application as an immunosensor. Biosens Bioelectron. 2003;19(3):185–92.
6. Dou XW, Zhang L, Liu C, Li Q, Luo J, Yang MH. Fluorometric competitive immunoassay for chlorpyrifos using rhodamine-modified gold nanoparticles as a label. Microchimca Acta. 2017;185(1):41.
7. Kim Young AH, Lee EH, Kim Kwang OK, Lee YT, Hammock BD, Lee HS. Competitive immunochromatographic assay for the detection of the organophosphorus pesticide chlorpyrifos. Anal Chim Acta. 2011;693:106–13.
8. Xu ZL, Wang Q, Lei HT, Eremin SA, Shen YD, Wang H, et al. A simple, rapid and high-throughput fluorescence polarization immunoassay for simultaneous detection of organophosphorus pesticides in vegetable and environmental water samples. Anal Chim Acta. 2011;708(1–2):123–9.
9. Sun Z, Wang W, Wen H, Gan C, Lei H, Liu Y. Sensitive electrochemical immunoassay for chlorpyrifos by using flake-like Fe_3O_4 modified carbon nanotubes as the enhanced multienzyme label. Anal Chim Acta. 2015;899:91–9.
10. Daems D, Lu J, Delport F, Marien N, Orbie L, Aernouts B, et al. Competitive inhibition assay for the detection of progesterone in dairy milk using a fiber optic SPR biosensor. Anal Chim Acta. 2017;950:1–6.
11. Shi S, Wang L, Su R, Liu B, Huang R, Qi W, et al. A polydopamine-modified optical fiber SPR biosensor using electroless-plated gold films for immunoassays. Biosens Bioelectron. 2015;74:454–60.
12. Zhang P, Chen Y-P, Wang W, Shen Y, Guo J-S. Surface plasmon resonance for water pollutant detection and water process analysis. TrAC Trends Anal Chem. 2016;85:153–65.
13. Gao YM, Zou F, Wu BP, Wang XX, Zhang JJ, Koh K, et al. CB[7]-mediated signal amplification approach for sensitive surface plasmon resonance spectroscopy. Biosens Bioelectron. 2016;81:207–13.
14. Fen YW, Yunus WM, Talib ZA, Yusof NA. Development of surface plasmon resonance sensor for determining zinc ion using novel active nanolayers as probe. Spectrochim Acta A. 2015;134:48–52.
15. Masson JF. Surface plasmon resonance clinical biosensors for medical diagnostics. ACS Sens. 2017;2(1):16–30.
16. Genslein C, Hausler P, Kirchner EM, Bierl R, Baeumner AJ, Hirsch T. Graphene-enhanced plasmonic nanohole arrays for environmental sensing in aqueous samples. Beilstein J Nanotech. 2016;7:1564–73.
17. Vaisocherova-Lisalova H, Visova I, Ermini ML, Springer T, Song XC, Mrazek J, et al. Low-fouling surface plasmon resonance biosensor for multi-step detection of foodborne bacterial pathogens in complex food samples. Biosens Bioelectron. 2016;80:84–90.

18. Liu X, Hu Y, Zheng S, Liu Y, He Z, Luo F. Surface plasmon resonance immunosensor for fast, highly sensitive, and in situ detection of the magnetic nanoparticles-enriched Salmonella enteritidis. Sensors Actuators B Chem. 2016;230:191–8.
19. Shankaran DR, Gobi KV, Miura N. Recent advancements in surface plasmon resonance immunosensors for detection of small molecules of biomedical, food and environmental interest. Sensors Actuators B Chem. 2007;121(1):158–77.
20. Feng L, Zhu A, Wang H, Shi H. A nanosensor based on quantum-dot haptens for rapid, on-site immunoassay of cyanotoxin in environmental water. Biosens Bioelectron. 2014;53:1–4.
21. Li S, Wu Q, Ma P, Zhang Y, Song D, Wang X, et al. A sensitive SPR biosensor based on hollow gold nanospheres and improved sandwich assay with PDA-Ag-Fe3O4-rGO. Talanta. 2018;180:156–61.
22. Shynkarenko OV, Kravchenko SA. Surface plasmon resonance sensors: methods of surface functionalization and sensitivity enhancement. Theor Exp Chem. 2015;51(5):273–92.
23. Vashist SK, Dixit CK, MacCraith BD, O'Kennedy R. Effect of antibody immobilization strategies on the analytical performance of a surface plasmon resonance-based immunoassay. Analyst. 2011;136(21):4431–6.
24. Lu B, Smyth MR, O'Kennedy R. Oriented immobilization of antibodies and its applications in immunoassays and immunosensors. Analyst. 1996;121:29–32.
25. Mauriz E, Calle A, Lechuga LM, Quintana J, Montoya A, Manclús JJ. Real-time detection of chlorpyrifos at part per trillion levels in ground, surface and drinking water samples by a portable surface plasmon resonance immunosensor. Anal Chim Acta. 2006;561(1–2):40–7.
26. Thepudom T, Lertvachirapaiboon C, Shinbo K, Kato K, Kaneko F, Kerdcharoen T, Baba A. Surface plasmon resonance-enhanced photoelectrochemical sensor for detection of an organophosphate pesticide chlorpyrifos. MRS Commun. 2017;8(01):107–12.