



An aptamer based surface plasmon resonance biosensor for the detection of ochratoxin A in wine and peanut oil

Zhiling Zhu^{a,b}, Mengxue Feng^{a,b}, Limin Zuo^b, Zhentai Zhu^c, Fengwei Wang^c, Long Chen^a, Jinghua Li^b, Guangzhi Shan^{b,*}, Shi-Zhong Luo^{a,*,**}

^a Beijing Key Laboratory of Bioprocess, College of Life Science and Technology, Beijing University of Chemical Technology, Beijing 100029 China

^b Institute of Medicinal Biotechnology, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100050 China

^c State Key Laboratory of NBC Protection for Civilian, Beijing, China

ARTICLE INFO

Article history:

Received 19 August 2014

Received in revised form

20 October 2014

Accepted 27 October 2014

Available online 30 October 2014

Keywords:

OTA analysis

SPR

Aptamer

Wine analysis

Peanut oil analysis

ABSTRACT

Ochratoxin A (OTA), as a kind of chlorophenolic mycotoxin, exist widely in plant origin food and is harmful to human. Herein, a surface plasmon resonance (SPR) biosensor using an anti-OTA aptamer immobilized sensor chip was developed to measure ochratoxin A (OTA) quantitatively through a straightforward direct binding assay. The streptavidin protein as a crosslinker was immobilized onto the surface of a sensor chip and the biotin-aptamer was captured through streptavidin-biotin interaction. The biosensor exhibited a detection range from 0.094 to 100 ng/mL (linear range from 0.094 to 10 ng/mL) of OTA with a lower detection limit of 0.005 ng/mL. Detection of OTA in wine and peanut oil was further performed in the SPR biosensor using simple liquid–liquid extraction for sample pretreatments. Recoveries of ochratoxin A from spiked samples ranged from 86.9% to 116.5% and coefficients of variation (CVs) ranged from 0.2% to 6.9%. The developed methods in our studies showed good analytical performances with limits of detection much lower than the maximum residue limit, as well as a good reproducibility and stability.

© Elsevier B.V. All rights reserved.

1. Introduction

Ochratoxin A (OTA) is a secondary metabolite produced by a variety of fungi, with most *Aspergillus* and *Penicillium* species being the primary producers. The chlorophenolic mycotoxin OTA is recognized as potentially harmful to humans and animals. OTA is clearly an immunosuppressive agent, potently nephrotoxic, carcinogenic and teratogenic, and of major concern for human health (Harvey et al., 1992). Furthermore, it has been considered by the International Agency for Research on Cancer to be a potential carcinogen (group 2B) for human (IARC, 1993). OTA contaminates cereals and cereal products, coffee, beans, pork meat and meat products, milk and milk products, eggs, wine, and beer all over the world (Binder et al., 2007). It has been established that wine is the second major source of OTA dietary intake by the EU population, following the cereals (Miraglia and Brera, 2002). In addition, a few surveys on the natural occurrence of ochratoxin A on peanuts have been reported (Ediage et al., 2014; Magnoli et al.,

2007; Palencia et al., 2014; Sangare-Tigori et al., 2006). Therefore, maximum permitted levels of OTA have been defined by nations all over the world. Typically, the European Commission has established the maximum level of OTA at 5 ng/mL for unprocessed cereals, 3 ng/mL for products derived from unprocessed cereals, 10 ng/mL for coffee beans, and 2 ng/mL for wine.

Under the consideration of the universality and perniciousness of OTA, the development of reliable and rapid detection methods of OTA in food samples is of great practical significance for food safety. High-performance liquid chromatography (HPLC) with fluorescence detectors was widely adopted as the standard methods for OTA detection (Zimmerli and Dick, 1995). In addition, gas chromatography–mass spectrometry (Olsson et al., 2002), thin-layer chromatography (TLC) (Pittet and Royer, 2002), enzyme linked immunosorbent assay (ELISA) (Flajs et al., 2009) and immunochromatographic assays as lateral flow strips (Lai et al., 2009) also showed good performance for OTA detection. However, new methods that are more direct, highly sensitive have attracting attentions on defining strategies suitable for real-time on site analysis and adaptable to different complex matrix.

In our work, we have developed a new biosensor based on the surface plasmon resonance (SPR) properties of gold surfaces and the peculiarity of aptamers. SPR is a phenomenon that occurs in

* Corresponding author. Fax: +86 10 63017302.

** Corresponding author. Fax: +86 10 64438015.

E-mail addresses: shanguangzhi@imb.pumc.edu.cn (G. Shan), luosz@mail.buct.edu.cn (S.-Z. Luo).

thin conducting film at an interface between media of different refractive index and has emerged as a powerful technique in biological and chemical analyzes. Moreover, this technique has been widely used for quantitative analysis of various target biomolecules in food (Homola, 2008). The principles of concentration measurement with SPR are largely similar to established interaction methods such as ELISA and ITC. While the major advantages of SPR lie in the real-time, label-free, non-invasive aspects of the measurement over other methods. The SPR detection technology allows measurements to be made on colored or turbid samples, which made sample preparation much simplified than many other established techniques. More recently, a bifunctional protein crosslinker-based surface plasmon resonance (SPR) biosensor was developed for the sensitive and rapid detection of aflatoxin B1 (Afb1) (Park et al., 2014b). In 2012, Lu et al. demonstrated a SPR biosensor inhibition immunoassay for determination of ractopamine (Rac) residue in pork (Lu et al., 2012). In most detection methods based on SPR, analyte-specific antibodies are used as recognized molecules. However, the production of specific Abs is difficult, expensive and extremely time-consuming, and antibodies can easily lose their activities when environmental conditions change.

In the present study, aptamer was used as a replacement of antibody for the detection of OTA. Aptamers are single-stranded oligonucleotides selected by an in vitro selection process named Systematic Evolution of Ligands by Exponential Enrichment (SELEX) (Ellington and Szostak, 1990; Mairal et al., 2008). For the development of biosensors and other analytical methods, aptamers were considered as an available alternative to antibodies or other bio-mimetic receptors (Tombelli et al., 2005). Unlike antibodies, they can be chemically derivatized easily to extend their lifetimes and bioavailability. With respect to antibodies, aptamers have the following advantages: low cost, little or no batch-to-batch variation and easy modification (Jayasena, 1999). The aptamer selected for OTA exhibits a high level of binding affinity specificity, showing a 100-fold less affinity to ochratoxin B (OTB), the dechlorinated analog of OTA (Cruz-Aguado and Penner, 2008). Based on these virtues, the aptamer functionalized SPR biosensor is developed for OTA detection in our studies.

Since the sensitivity of SPR sensors is determined by changes in the mass concentration of material at the surface, direct assays for small molecules are not routine in SPR biosensors. To obtain optimal sensitivity, most SPR assays use indirectly competitive or inhibition assays as well as high mass label methods. Compared to these indirect assays, direct assay has some attractive traits, such as increased speed of analysis, decreased use of biological reagents, and improved confidence in the detection of the small molecules, along with the ability to characterize the ligand/analyte (Yakes et al., 2014). With advances in SPR instrumentation, direct detection of small molecules is feasible with better sensitivity, and some SPR assays for direct detection of small molecules have been established in recent years (Yakes et al., 2014; Munoz et al., 2011).

Our study focused on developing a SPR biosensor for OTA detection using a straightforward direct binding assay. By optimization of experimental conditions, the biosensor exhibited a wide detection range from 0.094 to 100 ng/mL (linear range from 0.094 to 10 ng/mL) of OTA. Compared with the other SPR based biosensors for OTA detection, so far our SPR biosensor gives highest sensitivity with low LOD of 0.005 ng/mL. Besides, the biosensor was successfully used for the determination of OTA in wine and peanut oil samples, and could be further applied for determination of OTA in food matrixes.

2. Materials and methods

2.1. Reagents and instrumentation

Ochratoxin A (OTA, from *Aspergillus ochraceus*), Ochratoxin B (OTB, from *Aspergillus ochraceus*), Aflatoxin B1 (from *Aspergillus flavus*), Ochratoxin A ELISA Kit were purchased from Proibolab (Singapore). A 2 mg/mL stock solution of Ochratoxin A was prepared in dimethyl sulfoxide (DMSO, BioReagent, for molecular biology) and stored at -20°C . Kanamycin A (KanA) was purchased from National Institutes for Food and Drug Control. Streptavidin (SA) and N-acetyl-L-phenylalanine (Ac-Phe-OH) were purchased from Sigma Aldrich (China). Working standards were freshly prepared from concentrated stock by sequential dilution in Tris-HCl buffer (10 mM TRIS, pH 8.5, 120 mM NaCl, 5 mM KCl, and 20 mM MgCl_2). Amine Coupling Kit (contains 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS), 1.0 M Ethanolamine-HCl pH 8.5), 10 mM sodium acetate pH 5.0, 10 mM sodium acetate pH 4.5, 10 mM sodium acetate pH 4.0 were purchased from GE Healthcare. All components of buffers were obtained from Sigma-Aldrich. All oligonucleotides were synthesized by Sangon Biotech (Shanghai, China), and their sequences were anti-OTA aptamer: 5'-GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA-3'/Biotin. (Cruz-Aguado and Penner, 2008) Standardized wine and peanut oil samples were purchased from a local supplier (Beijing, China).

The Isothermal Titration Calorimeter (MicroCal™ iTC₂₀₀), SPR biosensor (BIAcore T200) and SPR sensor chips were obtained from GE Healthcare Bio-Sciences AB (America). ITC measures the heat change that occurs in two substrates interacting. BIAcore T200 is a high performance system for real-time biomolecular interaction analysis, using surface plasmon resonance technology (SPR). The detection occurs on the surface of the sensor chip (CM5), which forms one wall of the flow cell when the sensor chip is docked on to the ITC. For all experiments, the machine was purged with running buffer prior to analysis. All solutions were prepared using ultrapure water obtained from a Milli-Q ultrapure water system.

2.2. Isothermal titration calorimetry assay

ITC was performed using a MicroCal iTC₂₀₀ instrument. All samples were degassed and tempered before measurement. To ensure the buffers matching, two kinds of samples were dissolved in the same buffer (10 mM Tris, 20 mM MgCl_2 , 120 mM NaCl, 5 mM KCl and 2% DMSO, pH 8.5). The titration experiments were performed in a sample cell (300 μL) containing 8 μM DNA aptamer and a syringe (40 μL) containing 99 μM OTA. The reference power was set to 8 $\mu\text{cal/s}$, using a syringe stirring speed of 1000 rpm. The thermal equilibration step at 25°C was followed by an initial 60 s delay step. The standard binding experiments consisted of 16 successive 2.0 μL injections every 180 s; the first injection was 0.4 μL . The change in heat rate during the titration steps was registered in real time. Blank experiment was carried out under the identical operation conditions by directly injecting OTA solution into the buffer. Data were fit to a one set of sites binding model using the calorimeter software.

2.3. Preparation of the chip surface

SPR measurement was performed using a BIAcore T200 instrument with four flow channels and a sensor chip CM5 with dextran matrix. Streptavidin was attached to the dextran matrix using amine coupling method. pH 7.4 PBS buffer was used as a running buffer at a flow rate of 10 $\mu\text{L/min}$. The covalent immobilization of SA was carried out using a EDC/NHS method for

activating. In detail, a solution of 0.1 M N-hydroxysuccinimide (NHS) and 0.4 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was mixed (1:1) and 200 μL was injected onto channels 1 and 2 for 420 s to activate the carboxylic acid groups. 50 $\mu\text{g}/\text{mL}$ SA diluted with acetate buffer (pH 4.5) was loaded on the activated sensor chip. Finally, the remaining unreacted ester groups were blocked using 1 M ethanol amine (pH 8.5). The online response was used to characterize the sensor surface. Each step, including activation, immobilization, and blocking was carried out for a suitable time (Fig. 2A). For the biotin tagged aptamer, the flow rate was set to 5 $\mu\text{L}/\text{min}$. 10 μM biotin tagged aptamer diluted with PBS buffer was injected and captured onto the surface of channel 2 only.

2.4. Surface plasmon resonance assay

For the OTA assay, the reaction temperature was controlled at 25 ± 0.1 $^{\circ}\text{C}$. The data acquisition software was Biacore T200 Evaluation Software Version 2.0. With this biacore technology, the SPR angle change is reported as response units (RU). An SPR response of 1000 RU corresponds approximately to a surface concentration of 1 ng/mm^2 for average protein ligand on Sensor Chip CM5. All buffers for experiments were filtered (0.22 μm). The flow cells were arranged in pairs (Fc1-2 and Fc3-4) with minimum dead volume between the flow cells in a pair to provide accurate reference subtraction. The response obtained from the detection channel (Fc2 or Fc4) was normalized by subtracting the signal simultaneously acquired from the control channel (Fc1 or Fc3), which could eliminate nonspecific binding and buffer-induced bulk refractive index changes.

pH 8.5 Tris–HCl buffer, containing 10 mM TRIS, 120 mM NaCl, 5 mM KCl, and 20 mM MgCl_2 , was used as running buffer at a rate of 25 $\mu\text{L}/\text{min}$. Different concentrations of standard OTA (0–100 ng/mL) were prepared by serial dilutions in Tris–HCl from stock solution of 2 mg/mL OTA in DMSO. Of particular note was the DMSO content of OTA standard solution needed to be on the same.

The solutions were then injected onto the chip surface for 150 s at a flow rate of 25 $\mu\text{L}/\text{min}$. The binding between the captured aptamer and OTA was monitored in real-time. After each binding reaction, a further dissociation time of 300 s was applied after each injection to allow the signal back to the baseline. Report points were set at 4 s in relation to the end of injections in the cycle. At least 3 samples with the same concentration of OTA were detected.

2.5. Development of a simple sample extraction method

Two kinds of food were detected to validate the biosensor performance. The red wine and peanut oil samples without OTA were all purchased from local markets. The preparation of wine samples was established according to the related literature, with some simple modification (Yang et al., 2012). In detail, the red wine was spiked with the stock solution of OTA to obtain a final concentration of 100 ng/mL . From this spiked sample, several dilutions (0, 3, 40, 75 ng/mL) were prepared with non-spiked red wine. Spiked samples were equilibrated 2 h before extraction. Then, the red wine samples were mixed with the same volume of toluene. After overnight extraction, a given amount from each top phase was taken out for mixing with the same volume of running buffer. After complete phase separation, a given amount of each top phase was taken and used for detection. Peanut oil samples previously spiked with OTA at a final concentration of 0, 3, 40 and 75 ng/mL . Then, by vigorous shaking and standing for a certain length of time, the OTA was fully dissolved in oil. Afterwards, each oil sample was mixed with the same volume of running buffer and ultrasonic treated for 90 min. Then the oil samples were centrifuged and the bottom solution was transferred to a new vial and filtered through a 0.22 μm filter. 360 μL of the final solution was used to detection.

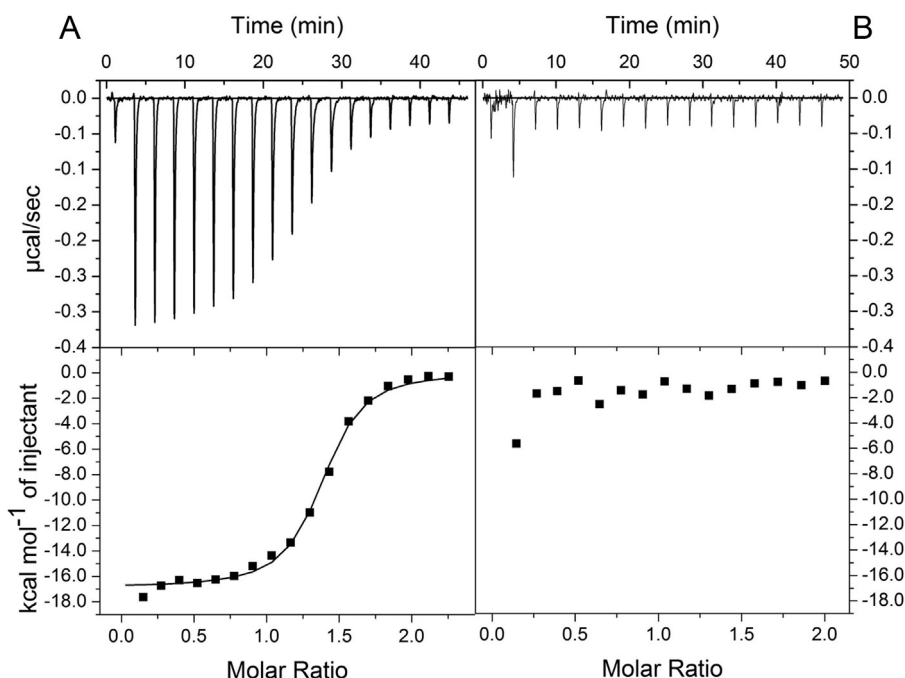


Fig. 1. ITC data curves and binding isotherms for titration of OTA with aptamer. (A) The binding affinity between OTA and aptamer in 10 mM Tris, pH 8.5, 120 mM NaCl, 5 mM KCl, 2% DMSO, and 20 mM Mg^{2+} . (B) The binding buffer without Mg^{2+} .

3. Results and discussions

3.1. Isothermal titration Calorimetry (ITC) assay

To confirm binding of OTA to aptamer marked by biotin, ITC experiments were performed and Fig. 1A (the top panel) shows the raw data from ITC measurements. All data could be fitted using the one-site model within the Origin software (Fig. 1A, the bottom panel), and giving an apparent equilibrium constant $K_A = 8.83 \times 10^6 \pm 1.20 \times 10^6$ nM with the Aptamer/OTA stoichiometry of 1.34 ± 0.01 . Our data showed a strong binding event between the OTA and biotin-aptamer and were consistent with the result reported in literature (Cruz-Aguado and Penner, 2008). Meanwhile, previous studies have reported that divalent cations influence the conformational structure of the aptamer, and binding of OTA to the DNA aptamer needs the presence of divalent ions (Mg^{2+} , Ca^{2+}) (Chung and Kwong, 2007). From the ITC experiment curve shown in Fig. 1B, it could be easily observed that OTA did not exhibit binding to the aptamer in the absence of magnesium ions. Presumably, it was suggested that the reliance on Mg^{2+} was due to a bridging interaction mediated by magnesium between the target and the oligonucleotide. Therefore, magnesium ions were added into the follow-up detection works.

3.2. Optimization and assessment of the OTA biosensor

In this assay, the streptavidin protein as a cross-linker was immobilized onto the surface of a CM5 sensor chip using a standard amine coupling protocol (Fischer, 2010). With this method, the dextran matrix on the sensor chip surface was first activated with a mixture of EDC and EHS to give reactive succinimide esters. SA was then passed over the surface and the esters reacted spontaneously with uncharged amino groups or other nucleophilic groups to link the SA covalently to the dextran (Fig. 2A). Streptavidin immobilized on the sensor chip surface can be used to capture biotinylated aptamer with high efficiency (K_D of the order of 10^{-15} M). The sensorgram for the immobilization of streptavidin and the capture of the biotin-aptamer were shown in Fig. 2 with an ideal result (SA, 2800 RU; aptamer, 1400 RU).

Surface plasmon resonance is a phenomenon that occurs in thin conducting films at an interface between media of different refractive index. The SPR signal is a direct measurement of the angle of minimum reflected intensity which is directly proportional to the mass concentration of the analyte. The ssDNA aptamer interacting partner was captured on the surface of the sensor chip through the modified biotin at the 5' end of the aptamer. The specific interaction of the aptamer with OTA was made use of biosensor assaying for the detection of OTA. Before the OTA determination, the performance of the biosensor was optimized. Instead of PBS buffer with Mg^{2+} , Tris-HCl buffer

(10 mM TRIS, pH 8.5, 120 mM NaCl, 5 mM KCl, and 20 mM $MgCl_2$) was chosen as the running buffer, since phosphate deposition can seriously disturb the detection or in the worst case block the flow system. The response increased with the sampling time and reached the steady value in 150 s. According to our results (Fig. 3A), the OTA bound to the aptamer with a fast dissociation rate constant and the relative response return to the baseline at 300 s after sample injection. This simple procedure makes it not necessary to establish a regeneration project. Meanwhile, we decided to use a flow rate of 25 μ L/min according to the experience of LMW (low molecular weight compound) experiments. Moreover, the specificity of the SA cross-linker based SPR biosensor for detection of OTA was checked by respectively introducing OTA analogs (ochratoxin B, and N-acetyl-L-phenylalanine), Kanamycin A and Aflatoxin B1 on the aptamer/anti-OTA layered chip. As the results presented in Fig. 3B, OTA (30 ng/mL) gave a relative response of 18.4 RU, which was obviously much greater than those of the other small molecules which gave responses < 2 RU. Furthermore, OTA (30 ng/mL) in the presence of 3000 ng/mL N-acetyl-L-phenylalanine (100 times higher concentration) yielded SPR signals of 17.6 RU, which is comparable with the OTA only (18.4 RU). These results demonstrate that OTA could elicit a measurable and significant response even in the OTA analog (Ac-Phe-OH), which further proves that the aptamer biosensor is specific for OTA detection.

To reduce the bulk contribution, the refractive index of samples and running buffer (Tris-HCl with 0.005% DMSO) should be carefully matched. In detail, the OTA stock (2 mg/mL in 100% DMSO) solution was diluted 20000 times to obtain a DMSO concentration of 0.005% using Tris-HCl buffer without DMSO. The assay running buffer (Tris-HCl with 0.005% DMSO) was then used to prepare a further dilution samples. To detect OTA using the aptamer based SPR biosensor, time dependent response changes at different concentration of OTA, in the range of 0.094–100 nM, under the optimized experimental conditions were recorded. As shown in Fig. 4A, an increased amount of OTA caused an increase of SPR response. From the sensorgram, it was found that the bulk contribution was weak due to the careful buffer match.

Considering analyte that dissociate rapidly from the surface, the relative response was measured at 146s which is right before the end of the injection. Each sample with different concentration was injected in triplicate and a calibration plot of the relative response at 146s was plotted against OTA concentration (B). Evaluation of concentration measurements used a fully defined four-parameter equation in biacore for fitting the curve to the calibration data points. Four curve parameters were obtained by the fitting function. The equation for the 4-parameter fit is $y = R_{hi} - (R_{hi} - R_{lo}) / (1 + (x/A_1)^{A_2})$. Where y and x are the plot coordinates, $R_{hi} = 6.049$ and $R_{lo} = 0.092$ are fitting parameters that correspond to the maximum and minimum response levels

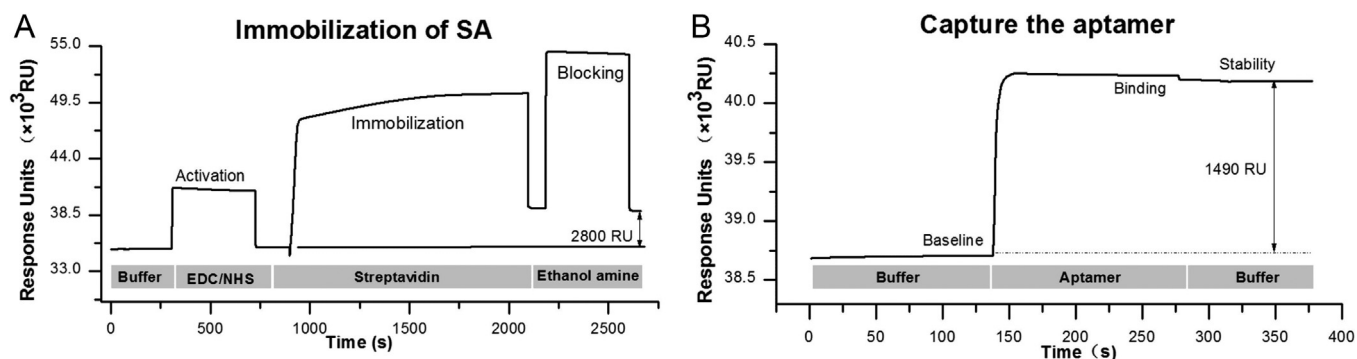


Fig. 2. Sensorgram for the immobilization of streptavidin and the capture of the biotin tagged aptamer on a CM5 chip. (A) Activation, 480 s; immobilization, 1200 s; blocking, 480 s; flow rate, 10 μ L/min; result, 2800 RU. (B) Capturer, 150 s; flow rate, 10 μ L/min; result, 1490 RU.

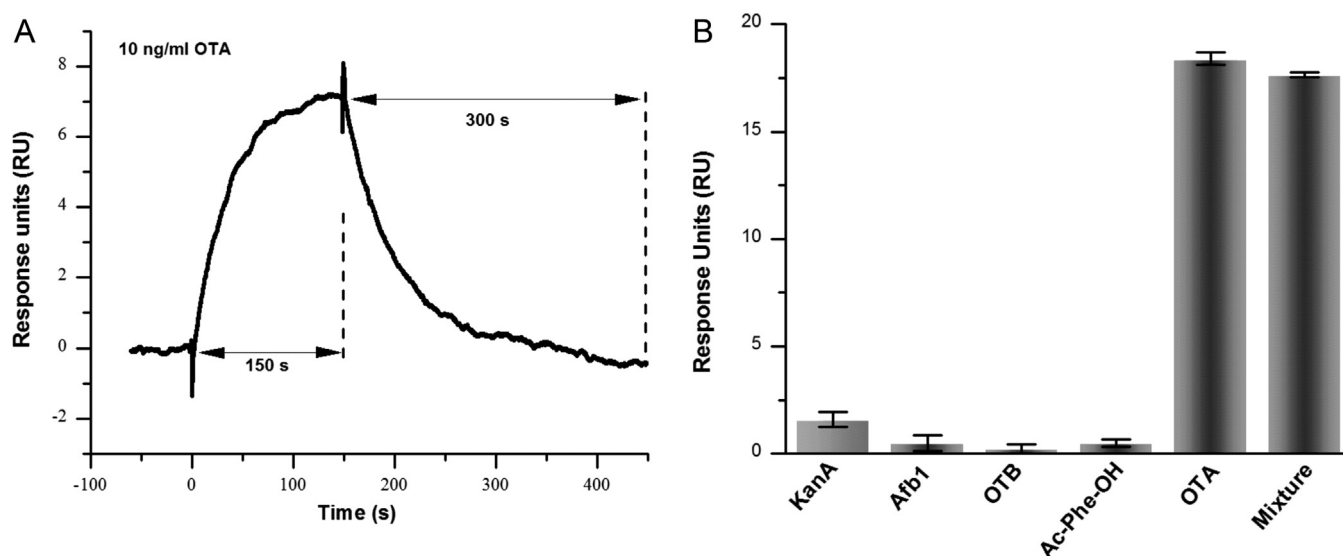


Fig. 3. Optimization of conditions. (A) Kinetics of the association and dissociation between surface-tethered aptamer and 10 ng/mL ochratoxin A in solution, expressed as variation of response with time. (B) Specificity of the sensor chip surface toward different compounds: KanA, Afb1, OTB, Ac-Phe-OH (each of 30 ng/mL); the mixture: Ac-Phe-OH (3000 ng/mL) and OTA (30 ng/mL).

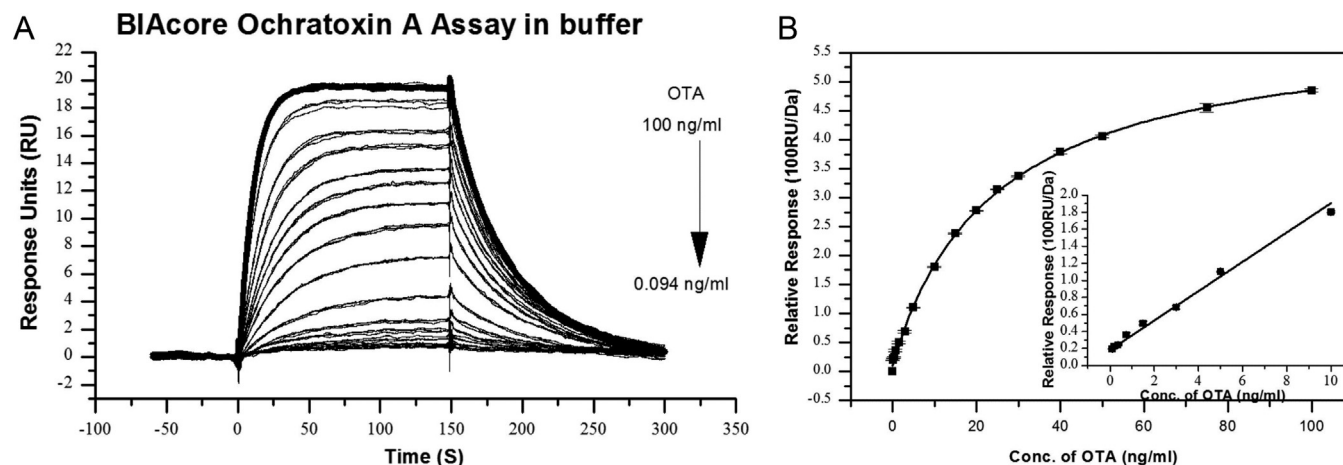


Fig. 4. A. Response plot for the aptamer based OTA SPR biosensor. Injection of OTA (0.094–100 ng/mL, flow rate: 25 μ L/min). B Calibration plots for the aptamer based OTA SPR biosensor, data points are the average plus $\pm$ one standard deviation ($n=3$, LOD=0.005 ng/mL, S/N=3).

Table 1
Recovery studies of two samples at three levels by SPR ($n=3$ replicates)

Sample	Spiked (ng/mL)	Theoretical response of OTA in buffer (RU)	Average response of OTA in spiked sample (RU) $\pm$ SD (RU)	Absolute recovery study (%)	CV (%)
Red wine	3	0.6830	0.7960 ± 0.0553	116.54	6.95
	40	3.7867	3.2977 ± 0.0724	87.09	2.20
	75	4.5550	3.9590 ± 0.0286	86.92	0.72
Peanut oil	3	0.6746	0.7325 ± 0.0305	108.59	4.17
	40	3.1670	2.7793 ± 0.0060	87.76	0.22
	75	3.2940	3.6087 ± 0.0402	91.95	1.11

respectively, $A_1=24.46$ and $A_2=0.982$ are additional fitting parameters, and the chi-squared value is 0.0037. Moreover, the biosensor exhibited a linear detection range from 0.094 to 10 ng/mL of the OTA and the good agreement ($r^2=0.9976$, Fig. 4 B). The limit of detection (LOD) is calculated to be 0.005 ng/mL ($S/N=3$) where the noise level is the standard deviation of replicate measurements on blank samples ($n=8$). Compared with the other SPR

based biosensors for OTA detection (Table S1, Fu, 2007; Hu et al., 2014; Yu and Lai, 2005; Urusov et al., 2011; Yuan et al., 2009; Park et al., 2014a; Zamfir et al., 2011; Yu and Lai, 2004), so far our SPR biosensor gives highest sensitivity with low LOD of 0.005 ng/mL. The repeatability of the biosensor was also evaluated by measuring the SPR responses of the same sample (10 ng/mL, OTA) six times, and a coefficient of variation (CV) of 1.7% was obtained, showing a good repeatability of the detection method.

3.3. Wine and peanut oil analysis by the biosensor

The OTA is a fatal toxin produced by several species of *Aspergillus* and *Penicillium* that grow in cereals, coffee, grape juice, beer and wine (Belli et al., 2002). In addition, it has been established in the literature that OTA does exist in the peanut (Ediage et al., 2014; Magnoli et al., 2007; Sangare-Tigori et al., 2006). Here, we assessed the feasibility of the analytical method in complex matrix samples by detecting OTA in red wine and peanut oil with the standard addition method. Sample preparation process is a critical step in the food safety testing. Currently, the most frequently used pre-treatment method, immunoaffinity column cleanup, encounter various shortcomings such as high cost and complicated operation

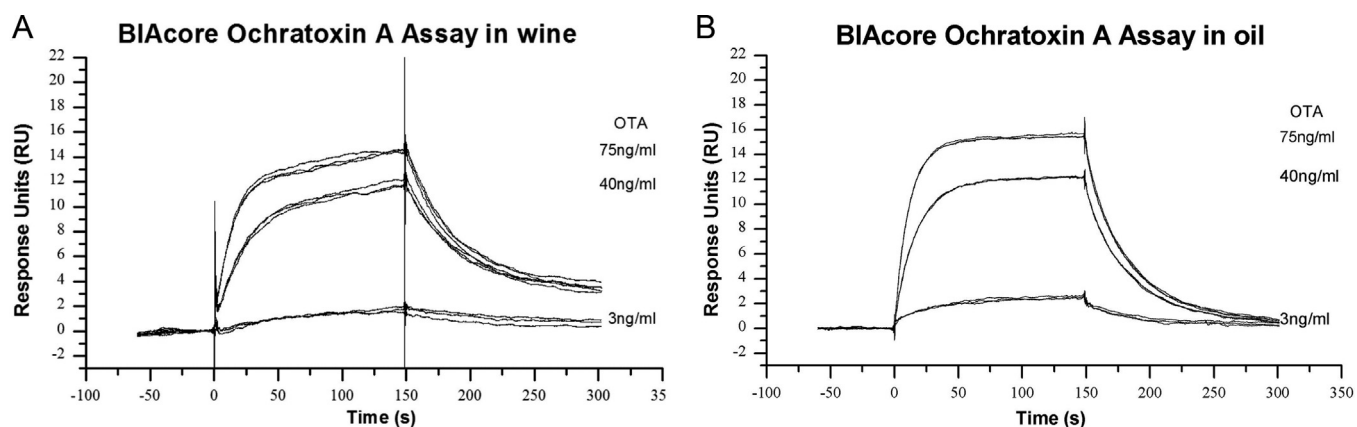


Fig. 5. Response plot for the aptamer based OTA SPR biosensor in spiked wine and oil samples and injection of OTA (3, 40, 75 ng/mL; flow rate: 25 μ L/min). (A) Biacore OTA assay in wine. (B) Biacore OTA assay in peanut oil.

in practical application (Castellari et al., 2000; Entwisle et al., 2001; Longobardi et al., 2013; Solfrizzo et al., 2008; Visconti et al., 2001; Zhao et al., 2014). In our research, two simple but efficient liquid–liquid extraction methods were developed for the separation of OTA from complex components existing in wine and peanut oil samples and we have successfully implemented the detection of oily substances through simple pretreatment. Experiments found that solubility of OTA in toluene was higher than that in wine (pH 3.0), but lower than that in running buffer (pH 8.5). In addition, solubility of OTA in running buffer was higher than that in peanut oil. Depending on this feature, extraction protocols of OTA from wine sample and peanut oil were developed and presented in Section 2.5. Free OTA were spiked in wine and peanut oil solutions with the final concentration of 3, 40, 75 ng/mL and mean absolute recoveries were analyzed to be 86.92–116.54% (Table 1). The sensorgram curves of spiked wine and peanut oil (Fig. 5) demonstrated that the coefficient of variation values were acceptable; indicating the biosensor had a promising feature for practical use in wine and peanut oil samples. The ELISA method was further performed and compared with our SPR assay. As shown in Table S2 and Fig. S1, a good correlation between SPR and ELISA method was obtained without any interference from real sample preparation process.

4. Conclusion

A biacore based aptamer biosensor was successfully established for the detection of OTA in two food complex matrices using a straightforward direct binding assay. The system relies on the principle of SPR, whose major difference from other methods lies in the real-time, label-free aspects of the measurement. Another advantage inherent in the detection method is that the measurement monitors each binding step in the assay procedure, not only the end-point level of the final interactant. The CM5 sensor chip was immobilized with the biotin-aptamer using a cross-linker of streptavidin. Due to the high affinity of streptavidin for biotin and the stable property of ssDNA, the deviation is very small, and the limit of detection (LOD) was one order of magnitude lower than those specified by E.U. legislation concerning limit of exposure in food. Importantly, the biosensor system can be easily regenerated by washing about 300 s. Finally, the actual application of the sensing system was quantified by determination of OTA in spiked wine and peanut oil samples. This study holds great promise for rapid and simple detection of micromolecule toxins. The study also offers two easy-to-use methods (SPR and ITC), by which the affinity of aptamer for their targets can be quantified.

Acknowledgement

This work was supported by the National Basic Research Program of China (973 program) (2013CB910700), the National Natural Science Foundation of China (21372026), the State Key Laboratory of NBC Protection for Civilian (No. SKLNBC2013-02K), Beijing NOVA Program (Z131102000413010), the National Natural Science Foundation of China (81302815).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.bios.2014.10.059>.

Reference

- Belli, N., Marin, S., Sanchis, V., Ramos, A.J., 2002. Food Sci. Technol. Int. 8, 325–335.
- Binder, E.M., Tan, L.M., Chin, L.J., Handl, J., Richard, J., 2007. Anim. Feed Sci. Technol. 137, 265–282.
- Castellari, M., Fabbri, S., Fabiani, A., Amati, A., Galassi, S., 2000. J. Chromatogr. A 888, 129–136.
- Chung, S.W., Kwong, K., 2007. J. AOAC Int. 90, 773–777.
- Cruz-Aguado, J.A., Penner, G., 2008. J. Agric. Food Chem. 56, 10456–10461.
- Ediage, E.N., Hell, K., De Saeger, S., 2014. J. Agric. Food Chem. 62, 4789–4797.
- Ellington, A.D., Szostak, J.W., 1990. Nature 346, 818–822.
- Entwisle, A.C., Williams, A.C., Mann, P.J., Russell, J., Slack, P.T., Gilbert, J., 2001. J. AOAC Int. 84, 444–450.
- Fischer, M.J., 2010. Amine coupling through EDC/NHS: a practical approach. In: Mol, N.J., Fischer, M.J. (Eds.), Surface Plasmon Resonance. Springer, New York, pp. 55–73.
- Flajs, D., Domijan, A.-M., Ivić, D., Cvjetković, B., Peraica, M., 2009. Food Control 20, 590–592.
- Fu, X.H., 2007. Anal. Lett. 40, 2641–2652.
- Harvey, R., Elisalde, M., Kubena, L., Weaver, E., Corrier, D., Clement, B., 1992. Am. J. Vet. Res. 53, 1966–1970.
- Homola, J., 2008. Chem. Rev. 108, 462–493.
- Hu, W.H., Chen, H.M., Zhang, H.H., He, G.L., Li, X., Zhang, X.X., Liu, Y., Li, C.M., 2014. J. Colloid Interface Sci. 431, 71–76.
- IARC, 1993. Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. WHO Press, Lyon, p. 599.
- Jayasena, S.D., 1999. Clin. Chem. 45, 1628–1650.
- Lai, W., Fung, D.Y., Yang, X., Renrong, L., Xiong, Y., 2009. Food Control 20, 791–795.
- Longobardi, F., Iacovelli, V., Catucci, L., Panzarini, G., Pascale, M., Visconti, A., Agostiano, A., 2013. J. Agric. Food Chem. 61, 1604–1608.
- Lu, X., Zheng, H., Li, X.-Q., Yuan, X.-X., Li, H., Deng, L.-G., Zhang, H., Wang, W.-Z., Yang, G.-S., Meng, M., Xi, R.-M., Aboul-Enein, H.Y., 2012. Food Chem. 130, 1061–1065.
- Magnoli, C., Astoreca, A., Ponsone, M.L., Fernandez-Juri, M.G., Barberis, C., Dalcero, A.M., 2007. Int. J. Food Microbiol. 119, 213–218.
- Mairal, T., Özalp, V.C., Sánchez, P.L., Mir, M., Katakis, I., O'Sullivan, C.K., 2008. Anal. Bioanal. Chem. 390, 989–1007.
- Miraglia, M., Brera, C., 2002. Accessed at: (http://europa.eu.int/comm/food/fs/scoop/3.2.7_en.pdf).

- Munoz, E.M., Lorenzo-Abalde, S., Gonzalez-Fernandez, A., Quintela, O., Lopez-Rivadulla, M., Riguera, R., 2011. *Biosens. Bioelectron.* 26, 4423–4428.
- Olsson, J., Börjesson, T., Lundstedt, T., Schnürer, J., 2002. *Int. J. Food Microbiol.* 72, 203–214.
- Palencia, E.R., Mitchell, T.R., Snook, M.E., Glenn, A.E., Gold, S., Hinton, D.M., Riley, R. T., Bacon, C.W., 2014. *J. Food Prot.* 77, 805–813.
- Park, J.H., Byun, J.Y., Mun, H., Shim, W.B., Shin, Y.B., 2014a. *Biosens. Bioelectron.* 59, 321–327.
- Park, J.H., Kim, Y.-P., Kim, I.-H., Ko, S., 2014b. *Food Control* 36, 183–190.
- Pittet, A., Royer, D., 2002. *J. Agric. Food Chem.* 50, 243–247.
- Sangare-Tigori, B., Moukha, S., Kouadio, H.J., Betbeder, A.-M., Dano, D.S., Creppy, E. E., 2006. *Food Addit. Contam.* 23, 1000–1007.
- Solfrizzo, M., Panzarini, G., Visconti, A., 2008. *J. Agric. Food Chem.* 56, 11081–11086.
- Tombelli, S., Minunni, M., Mascini, M., 2005. *Biosens. Bioelectron.* 20, 2424–2434.
- Urusov, A.E., Kostenko, S.N., Sveshnikov, P.G., Zherdev, A.V., Dzantiev, B.B., 2011. *Sens. Actuators B-Chem.* 156, 343–349.
- Visconti, A., Pascale, M., Centonze, G., 2001. *J. AOAC Int.* 84, 1818–1827.
- Yang, C., Lates, V., Prieto-Simon, B., Marty, J.L., Yang, X., 2012. *Biosens. Bioelectron.* 32, 208–212.
- Yakes, B.J., Kanyuck, K.M., DeGrasse, S.L., 2014. *Anal. Chem.* 86, 9251–9255.
- Yu, J.C., Lai, E.P., 2004. *Synth. Met.* 143, 253–528.
- Yu, J.C., Lai, E.P., 2005. *React. Funct. Polym.* 63, 171–176.
- Yuan, J., Deng, D.W., Lauren, D.R., Aguilar, M.I., Wu, Y.Q., 2009. *Anal. Chim. Acta* 656, 63–71.
- Zamfir, L., Geana, I., Bourigua, S., Rotariu, L., Bala, C., Errachid, A., Jaffrezic-Renault, N., 2011. *Sens. Actuators B-Chem.* 159, 178–184.
- Zhao, X., Yuan, Y., Zhang, X., Yue, T., 2014. *Food Control* 46, 332–337.
- Zimmerli, B., Dick, R., 1995. *J. Chromatogr. B Appl.* 666, 85–99.