Journal of Analytical Science and Technology

RESEARCH ARTICLE Open Access

One-step immunoassay based on switching peptides for analyzing ochratoxin A in wines

Tae-Hun Kim[1], Ji-Hong Bong[1], Hong-Rae Kim[1], Won-Bo Shim[2], Min-Jung Kang[3] and Jae-Chul Pyun[1*]

Abstract

A one-step immunoassay is presented for the detection of ochratoxin A (OTA) using an antibody complex with switching peptides. Because the switching peptides (fluorescence-labeled) were able to bind the frame region of antibodies (IgGs), they were dissociated from antibodies immediately when target analytes were bound to the binding pockets of antibodies. From the fluorescence signal measurements of switching peptides, a quantitative analysis of target analytes, via a one-step immunoassay without any washing steps, could be performed. As the first step, the binding constant (K_D) of OTA to the antibodies was estimated under the continuous flow conditions of a surface plasmon resonance biosensor. Then, the optimal switching peptide, among four types of switching peptides, and the reaction condition for complex formation with the switching peptide were determined for the one-step immunoassay for OTA analysis. Additionally, the selectivity test of one-step immunoassay for OTA was carried out in comparison with phenylalanine and zearalenone. For the application to the one-step immunoassay to detect OTA in wines, two types of sample pre-treatment methods were compared: (1) a liquid extraction was carried out using chloroform as a solvent with subsequent resuspension in phosphate-buffered saline (total analysis time < 1 h); (2) direct dilution of the wine sample (total analysis time < 0.5 h). Finally, the direct dilution method was found to be effective for the one-step immunoassay based on the switching peptide assay for OTA in wines with a markedly improved total analysis time (< 0.5 h). Additionally, the assay results were compared with commercial lateral flow immunoassay.

Keywords: One-step immunoassay, Switching peptide, Ochratoxin (OTA), Wine, SPR biosensor

Introduction

Ochratoxin A (OTA) is a well-known foodborne mycotoxin found in various types of agricultural products, from cereal grains to wine and coffee. OTA is produced by several types of fungi that have different optimal growing temperatures and water activities, such as *Aspergillus niger*, *A. ochraceus*, *A. carbonarius*, and *Penicillium verrucosum* (Bui-Klimke and Wu 2015). OTA contamination of agricultural products occurs owing to poor storage conditions and the drying process. As this compound has been reported to be toxic and carcinogenic to animals, maximum limits on foods and drinks have been proposed by the food safety authorities of many countries (Mateo et al. 2007; Bellver Soto et al. 2014; Ortiz-Villeda et al. 2021). As shown in Fig. 1a, the analysis of OTA has been most frequently carried out using high-performance liquid chromatography (HPLC) equipped with a mass spectrometer and fluorescence detectors (Kholová et al. 2020). Various types of solid-phase extraction and liquid–liquid extraction methods have been used for sample preparation in food control laboratories and routine practice (Visconti et al. 2001; Pena et al. 2005; Aresta et al. 2006). The analytical parameters of other methods for OTA analysis are summarized in Table 1 (Tessini et al. 2010; Liu et al. 2021; Gupta et al. 2017; Lv et al. 2017; Lv et al. 2014; Karczmarczyk et al. 2016; Anfossi et al. 2013; Khan and Dhayal 2008; Dai et al. 2016; Taradolsirithitikul et al. 2017; Zou et al. 2016).

*Correspondence: jcpyun@yonsei.ac.kr

[1] Department of Materials Science and Engineering, Yonsei University, 50 Yonsei-Ro, Seodaemun-Gu, Seoul 03722, Republic of Korea
Full list of author information is available at the end of the article

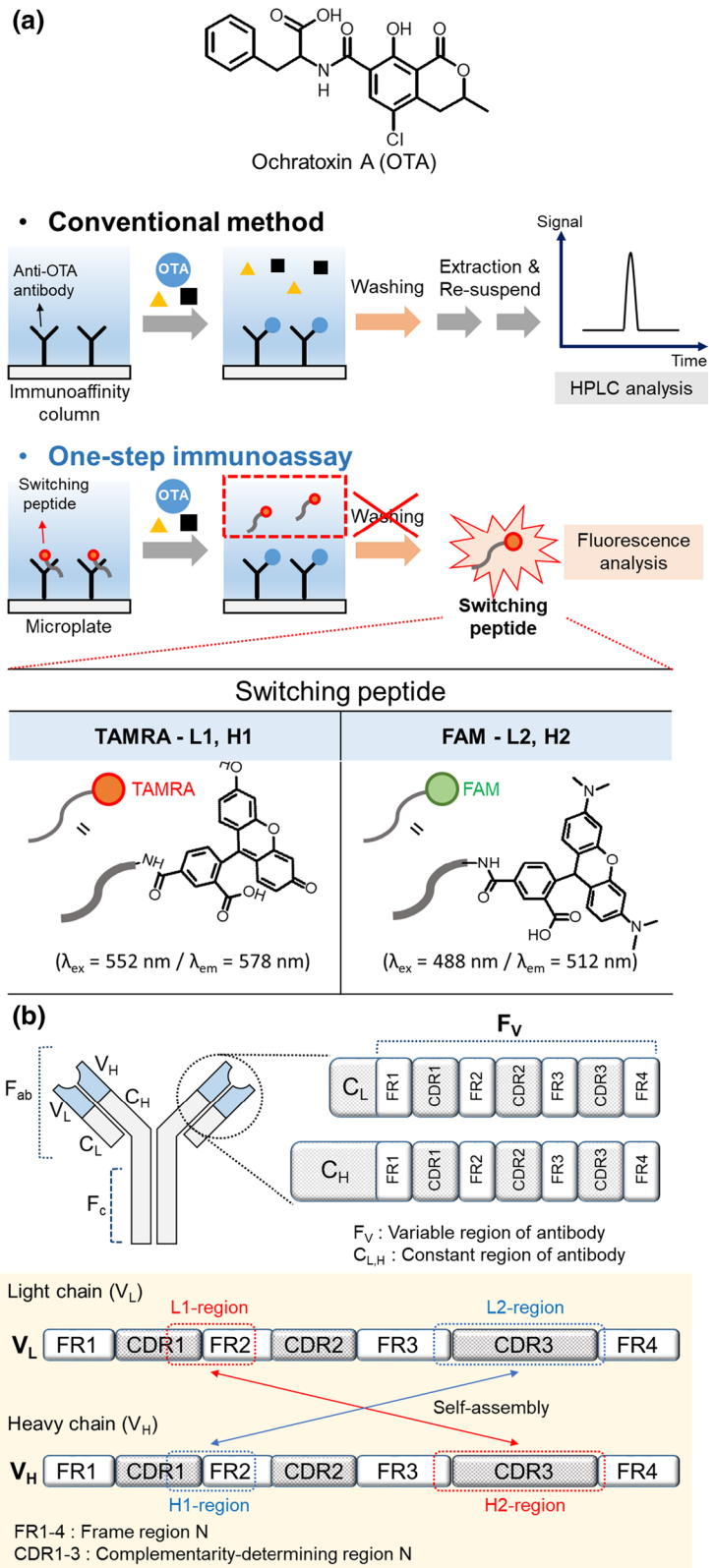

Fig. 1 One-step immunoassay based on switching peptides. **a** Comparison of OTA detection configurations using one-step immunoassay and conventional method. **b** Switching peptides that specifically bind to an antigen-binding pocket of IgG. Binding pocket of IgG composed of heavy and light chains with complementarity-determining regions (CDRs) and frame regions (FRs)

Table 1 Comparison of analytical methods for the detection of OTA

Quantification method	Type of food	Limit of density	Author	References
HPLC-FLD	Red wine	0.01 ng/mL	Tessini and others	Tessini et al. (2010)
MIP-based SPME by HPLC-FLD	Beer	0.07 ng/mL	Liu and others	Liu et al. (2021)
Differential pulse voltammetry based on amino-acid-functionalized ZrO_2	Coffee	0.3 ng/mL	Gupta and others	Gupta et al. (2017)
Aptamer based on AuNPs	Coffee	9.2 ng/mL	Xin and others	Lv et al. (2017)
Nanoparticle-enhanced SPR	Red wine	0.19 ng/mL	Karczmarczyk and others	Karczmarczyk et al. (2016)
Silver-enhanced lateral flow assay	Red wine, white wine	1.2 ng/mL	Anfossi and others	Anfossi et al. (2013)
Impedimetric based on TiO_2NPs	–	1 ng/mL	Khan and Dhayal	Khan and Dhayal (2008)
Luminescence resonance energy transfer-based aptasensor	Beer	27.0 pg/mL	Dai and others	Dai et al. (2016)
Label-free fluorescence aptamer	Beer	Below 1 ng/mL	Zhenzhen and others	Lv et al. (2014)
Fourier transform near-infrared spectroscopy	Green coffee beans	–	Taradolsirithitikul and others	Taradolsirithitikul et al. (2017)
ELISA based on biotinylated mimotope	Corn	Below 2.5 ng/mL	Zou and others	Zou et al. (2016)

In the present study, a one-step immunoassay based on switching peptides was applied for effective OTA analysis in wines with a far shorter analysis time than the HPLC method. One-step immunoassay methods can produce the assay result only by mixing analyte solutions, without any washing steps (Rong et al. 2019; Kaarj et al. 2020). Because the switching peptides (fluorescence-labeled) can bind to the frame regions of antibodies (immunoglobulin G [IgG]), these switching peptides are dissociated from antibodies immediately after target analytes (specific antigens) become bound to the binding pockets of antibodies. As shown in Fig. 1a, the one-step immunoassay uses an antibody complex with switching peptides before the analysis. When target analytes are incubated with the antibody complex, the bound switching peptides, with fluorescence capability, are dissociated in the solution, and a fluorescence signal is produced and can be quantitatively analyzed according to the amount of bound analytes. Therefore, the assay results could be reported just by mixing the analytes without any washing steps. The switching peptides are designed from the structure of IgG. The binding pocket region of IgG is generated via the self-assembly of frame regions (FRs) of the heavy chain (V_H) and light chain (V_L) (Bong et al. 2021; Park et al. 2022; Sung et al. 2022; Kim 2022a). As shown in Fig. 1b, the $L1$ (or $L2$) region at V_L interacts with the $H2$ (or $H1$) region of V_H. These regions can be chemically synthesized into switching peptides and can spontaneously bind to the corresponding regions. As IgGs have conserved amino acid sequences in these regions, the switching peptides made of the amino acid sequences of FRs can be used for any type of IgG. When the binding constant (K_D) of these switching peptides is lower than that of target analytes in the immunoaffinity interactions, the switching peptides bound to IgG are released immediately upon the binding of target analytes.

In this work, the one-step immunoassay based on switching peptide was presented. As the first step, the K_D of OTA to the antibodies and switching peptides was estimated using a surface plasmon resonance (SPR) biosensor. Then, the optimal switching peptide, among four types of switching peptides, and the reaction condition for complex formation with the switching peptide were determined for the one-step immunoassay for OTA analysis. Finally, the one-step immunoassay to detect OTA in wines was carried out using two types of sample pretreatment methods: (1) a liquid extraction was carried out using chloroform as a solvent and subsequent resuspension in PBS (total analysis time < 1 h) and (2) direct dilution of the wine sample (total analysis time < 0.5 h). Additionally, the assay results were compared with commercial lateral flow immunoassay.

Materials and methods

Materials

Bovine serum albumin (BSA), OTA, mercaptoundecanoic acid (MUA), *N*-ethylcarbodiimide (EDC), *N*-hydroxysuccinimide (NHS), ethanolamine, and chemical solvents of analytical grade were purchased from Sigma–Aldrich Korea (Seoul, Korea). MaxiSorp 96-well plates were purchased from SPL Life Sciences (Pocheon, Korea). The anti-OTA antibody was supplied by Gyeongsang National University (Jinju, Korea). Wine samples were prepared using commercially available products. Labeled peptides were synthesized by Peptron (Daejeon, Korea). A lateral flow immunoassay kit was purchased from Elabscience (Texas, USA).

Purification of *Z*-domain

Escherichia coli strain BL21(DE3) was used to purify the *Z*-domain. BL21(DE3) is a modified strain from BL21 that has the coding sequence of *T*7 RNA polymerase, which is required for the protein expression system based on the *T*7 promoter (Studier and Moffatt 1986). To obtain *Z*-domain-expressing cells, plasmid pJ349G was transformed into the *E. coli* via electroporation, and the strain was grown in high-salt Luria–Bertani (LB) medium (10 mL) containing kanamycin (50 mg/L, 10 µL) by shaking (200 rpm) for 16 h at 37 °C. For the main culture, *E. coli* (500 µL) was cultured again in high-salt LB medium (50 mL) with kanamycin (50 mg/L, 50 µL), 10 mM ethylenediaminetetraacetic acid (25 µL), and 99% β-mercaptoethanol (40 µL) at 37 °C with shaking (200 rpm) until an OD600 nm of 0.6 was reached (Yoo et al. 2011; Ueda et al. 2016). To induce *Z*-domain expression, cultured *E. coli* cells (50 mL, OD600 nm = 0.6) were incubated with 1 M isopropyl *β*-D-1 thiogalactopyranoside (50 µL) at 30 °C with agitation (125 rpm) for 2 h. The cultured *E. coli* BL21(DE3) cells (50 mL) were harvested by centrifugation at 7000 × *g* for 3 min and washed three times with PBS (10 mL). The cells were re-suspended in a binding buffer (5 mM Tris–HCl, 0.5 mM EDTA, and 1 M NaCl, 40 mL), and the ultrasonication was performed with an aliquot of *E. coli* culture solution (20 mL) with 120 repeated cycles, which consisted of 5 s of ultrasonication at a power of 20% and 5 s of incubation in ice, using a sonic reactor (Vibra cell VCX-130, Sonics, USA). After ultrasonication, the *E. coli* cells were centrifuged at 7000 × *g* for 5 min at 4 °C, and then, the supernatant was transferred to a 50-mL Eppendorf tube. Then, the *Z*-domain in the supernatant was purified using a His-tag purification column (Roche, Basel, Switzerland) and an elution buffer (binding buffer with 500 mM imidazole). Purified proteins were dialyzed to remove imidazole for 16 h at 100 rpm and 4 °C.

Sample pre-treatment

The actual sample test was performed using spiked samples of commercially available wine. The extraction of OTA from real samples was carried out using chloroform. For the extraction of OTA, the OTA-spiked wine samples, based on different concentrations (500 µL), were first vortex-mixed with chloroform (1 mL) for 30 s and then incubated for 10 min at room temperature. Thereafter, the chloroform layer was acquired and dried by centrifugation (1500 rpm for 1 h at 60 °C) using a rotary evaporator. Finally, the dried extracted sample was re-suspended in PBS (200 µL) for fluorescence analysis (Jesus et al. 2017). For the direct dilution method, the OTA from real samples was carried out using a binding buffer (5% $NaHCO_3$, 1% polyethylene glycol solvent). For the extraction of OTA, the OTA-spiked wine sample based on different concentrations (10 g) was first mixed with binding buffer to a 20 mL final volume and then homogenized for 5 min. Thereafter, the homogenized sample was filtered with Whatman No. 4 (12.0 cm) paper, and the wine samples were prepared through dilution with PBS.

HPLC assay

The quantitative analysis of OTA was carried out as a reference method using HPLC from Young-In Science Co (Seoul, Korea). For the HPLC analysis, a Kinetex C18 reversed-phase column from Phenomenex (Torrance, CA, USA) was used alongside acetonitrile with 0.1% trifluoroacetic acid (TFA) and distilled water (DW) with 0.1% TFA as the mobile phase. The flow rate was set to 0.5 mL/min. The standard OTA samples were prepared by spiking OTA in a concentration range of 1 to 1000 nM, and each sample was injected based on a volume of 100 µL. The mobile phase composition was programmed to be 100% DW for the first 5 min (0–5 min), and the acetonitrile percentage was increased up to 80% (5–21 min) via linear gradation. The OTA was detected using a UV detector at a wavelength of 333 nm.

SPR biosensor

BK-7 glass was coated with a Ti film (2 nm), followed by an Au layer (48 nm). The specific binding properties of the isolated antibodies with OTA were used for the quantitative analysis of OTA using an SPR biosensor. The isolated antibodies against OTA were immobilized on the SPR biosensor through covalent binding. The gold surface of the SPR biosensor was modified to incorporate carboxylic acid using MUA (1 mM). The anti-OTA antibodies (10 µg/mL) were covalently bound for 2 h using the EDC/NHS reagent, and then, the antibody-modified gold chip was blocked with

(See figure on next page.)

Fig. 2 Determination of affinity constants (K_D) of immobilized anti-OTA antibodies against OTA and switching peptides using SPR biosensor. **a** Covalent immobilization of anti-OTA antibody to the modified surface of SPR chip. **b** SPR sensorgram for the treatments of OTA and dose–response curve from SPR measurements at different concentrations of OTA against anti-OTA antibody. **c** SPR sensorgrams at different concentrations of switching peptides using immobilized anti-OTA antibodies and calculation of affinity constants (K_D) for each switching peptide against the same anti-OTA antibody

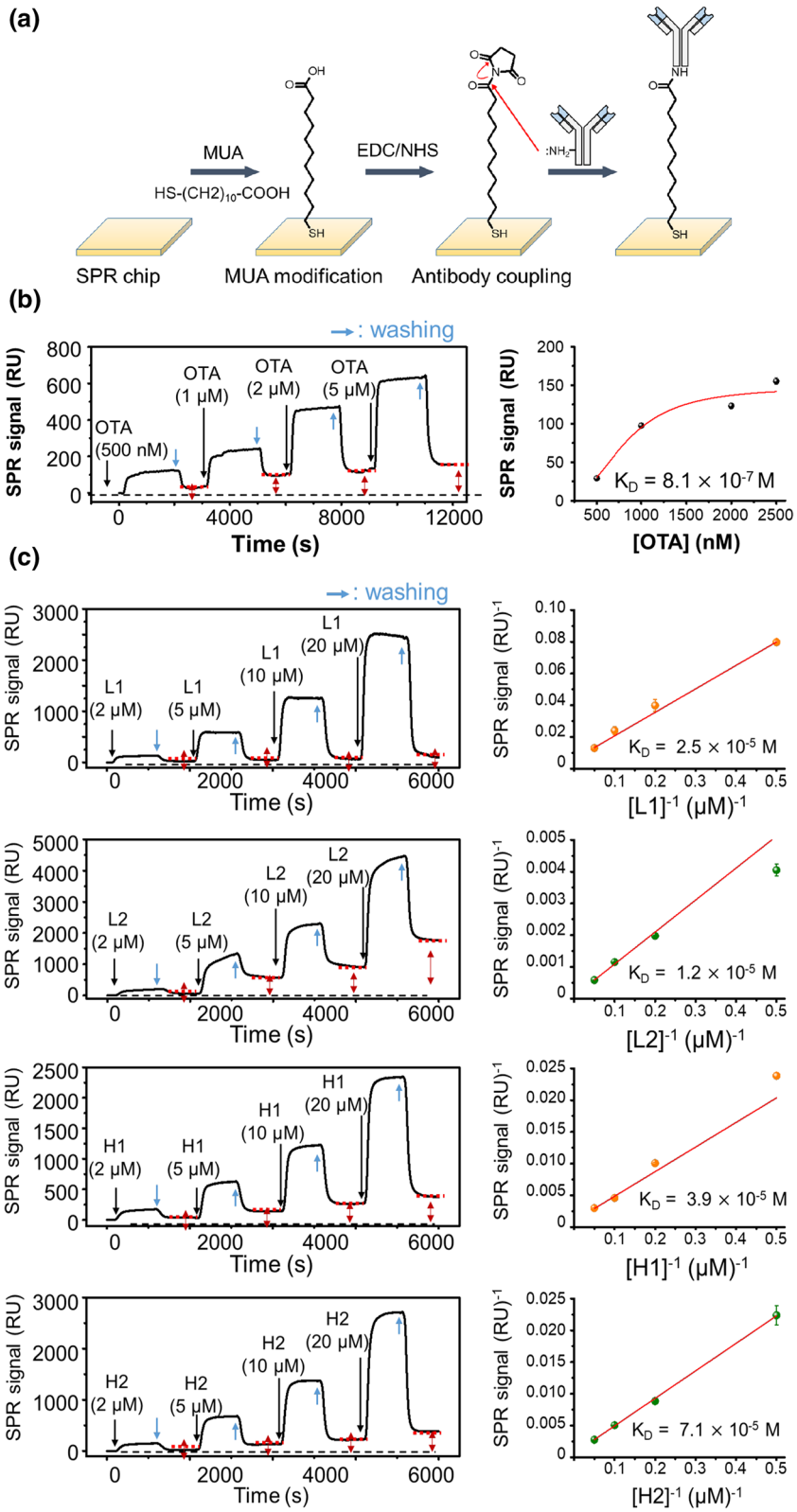

Fig. 2 (See legend on previous page.)

ethanolamine (1 M), as shown in Fig. 2a (Bong et al. 2020). The SPR signal for each sample was calculated as the change in the signal baseline prior to and after sample incubation using an SPR biosensor from i-ClueBio (Sungnam, Korea) (Bong et al. 2020; Jung et al. 2021a, b; Kim 2022b). The signal baseline was established from the averaged SPR signals following washing with PBS for 20 min at a flow rate of 20 µL/min. The SPR biosensors were prepared to separately inject samples of OTA and the four types of switching peptides. As shown in Fig. 2b, c, the SPR signal was measured for OTA samples at four different concentrations and samples of switching peptides labeled with FAM and TAMRA at four different concentrations. The dose–response curve for the samples was carried out by the isotherm model (shown below), where R (R_{MAX}) represents the SPR signal (maximum SPR signal), [Ag] represents the concentrations of OTA and switching peptides labeled with FAM and TAMRA, and K_D represents the affinity constant of the OTA and switching peptides labeled with FAM and TAMRA for the purified anti-OTA antibodies.

$$R = R_{MAX} \times \left([Ag]/[Ag] + K_D\right)$$

One-step immunoassay based on switching peptides

To evaluate specific binding to antibodies, a microplate-based assay was performed. The *Z*-domain (1 µM) was incubated in the microplate for 2 h at room temperature for antibody orientation. Then, the anti-OTA antibody (10 µg/mL) was incubated in the microplate for 2 h at room temperature. After blocking with 0.5% BSA prepared in PBS, the switching peptides (2 µM) were added to each well and incubated for 1 h. And then, OTA samples were added at four different concentrations and incubated for 1 h. Subsequently, the solutions were transferred directly into a new microplate, and the fluorescence intensity was measured using the Victor X5 fluorescence spectrometer from PerkinElmer Inc. (Waltham, Massachusetts, USA).

One-step immunoassay based on lateral flow assay

The lateral flow assay was carried out using a commercial lateral flow immunoassay kit from Elabscience (Texas, USA). The OTA samples were prepared by spiking OTA in a concentration range of 1 to 250 nM. For the lateral flow assay analysis, sample pre-treatment was performed as followed procedure: (1) Preparation of wine sample by mixing a white wine (2 g) and methanol (15 mL). (2) After centrifugation at 4000 rpm/min for 3 min, the supernatant (1 mL) was dried in a water bath at the temperature of 60 °C, and then resolved in reconstitution buffer (0.3 mL) in the kit. The lateral flow immunoassay was performed using a prepared wine sample according to the manufacturer's instructions.

Results and discussion

One-step immunoassay with switching peptides

One-step immunoassay methods can produce the assay result only by mixing analyte solutions, without any washing steps (Rong et al. 2019; Kaarj et al. 2020). Because the switching peptides (fluorescence-labeled) can bind to the frame regions of antibodies (immunoglobulin G [IgG]), these switching peptides are dissociated from antibodies immediately after target analytes (specific antigens) become bound to the binding pockets of antibodies. As shown in Fig. 1a, the one-step immunoassay uses an antibody complex with switching peptides before the analysis. When target analytes are incubated with the antibody complex, the bound switching peptides, with fluorescence capability, are dissociated in the solution, and a fluorescence signal is produced and can be quantitatively analyzed according to the amount of bound analytes. The bound analyte concentration was estimated based on the measurement of fluorescence from the released switching peptides (Rong et al. 2019; Kaarj et al. 2020). In this work, two kinds of fluorescence labels were used for four kinds of switching peptides: TAMRA for *L*1, *H*1 switching peptides and FAM for *L*2, *H*2 switching peptides. The fluorescence spectra for switching peptide *L*1 with a fluorescence label of TAMRA (λ_{ex} = 552 nm, λ_{ex} = 578 nm) and switching peptide *L*2 with a fluorescence label of FAM (λ_{ex} = 488 nm, λ_{ex} = 512 nm) presented in Additional file 1: Fig. S1. The preliminary conditions for this one-step immunoassay comprised the difference between the K_D of antibodies against switching peptides and that against target analytes (OTA). In other words, the binding of target analytes (OTA) should be stronger than that of the switching peptides. These binding properties of switching peptides and the target analyte (OTA) were estimated using an SPR biosensor. As shown in Fig. 2a, antibodies against OTA were covalently immobilized on the Au surface of the SPR biosensor using MUA (11 carbons). As the first step, the K_D of OTA to the antibodies was estimated by injecting analytes at different concentrations under continuous flow conditions. As shown in Fig. 2b, the K_D of OTA to the antibodies was estimated to be 8.1×10^{-7} M. As the next step, the binding constants of four types of switching peptides to the antibodies were estimated using the same conditions for SPR measurements. As shown in Fig. 2c, switching peptides *L*1, *L*2, *H*1, and *H*2 at four different concentrations were reacted with the immobilized antibodies on the SPR biosensor. As previously explained, the amino acid sequences of switching peptides were derived from the FRs of IgG, as shown in

Fig. 1b. As the FRs located at heavy and light chains of IgG are symmetrically bound, the switching peptides derived from these regions were expected to have similar binding constants, and the binding constant of switching peptides, from SPR measurements, was similar for different types of switching peptides. The K_D of OTA to the antibodies was estimated to be 2.5×10^{-5} M for switching peptide *L*1, 1.2×10^{-5} M for switching peptide *L*2, 3.9×10^{-5} M for switching peptide *H*1, and 7.1×10^{-5} M for switching peptide *H*2 as summarized in Table 2. These results showed that the binding constant of OTA was 100-fold lower than that with the four types of switching peptides, which indicated stronger binding of the target analyte (OTA) to the antibodies compared with that with the switching peptides. From these results, the binding properties of the target analyte (OTA) were determined to be suitable for the one-step immunoassay based on switching peptides.

Target analytes bind to antibodies through specific interactions within binding pockets. Three complementarity-determining regions (CDRs; CDR1, CDR2, and CDR3) are located at light and heavy chains, and the interaction with target analytes occurs with specific parts of these areas. The switching peptides bind frame regions between CDR regions, and the switching peptides dissociate through binding to target analytes. As the binding sites within the binding pocket of IgG may differ according to the target analytes, the dissociation of switching peptides could differ. To determine the optimal switching peptide for the detection of OTA, the dissociation rate of each peptide was determined with the same antibodies used against OTA. For measurements of the dissociation rate, the antibodies against OTA were bound to the switching peptide. As shown in Fig. 3a, the antibody and switching peptide complex was prepared by incubating four types of switching peptides, based on a concentration range of 1–5 μM, with the immobilized antibodies against OTA. Then, the dissociation of these four switching peptides was estimated after treatment with PBS (negative control) and OTA solution (1 μM). The optimal switching peptide should have a high dissociation ratio compared with that of PBS (negative control), and switching peptide *L*2 had the maximum dissociation ratio among all switching peptides. Additionally, switching peptide *L*2 (2 μM) showed a maximum dissociation ratio of 250% compared with that of PBS (negative control). In addition, switching peptide *H*2 (1 μM) had a dissociation ratio of 59% compared with that of PBS (negative control). These results showed that the FRs for the binding of switching peptides *L*2 and *H*2 are located close to the binding site of the target analyte, OTA. The optimal switching peptides *L*2 and *H*2 were then used for the quantitative analysis of OTA under each optimal dissociation condition. As shown in Fig. 3b, the one-step immunoassay based on 2 μM switching peptide *L*2 showed a linear correlation ($R^2 = 0.976$, $n = 3$) for OTA in a concentration range of 1 to 1000 nM, and the limit of detection (LOD) was estimated to be 0.60 nM ($n = 3$). When the antibody complex for the one-step immunoassay was generated by administering the switching peptide *L*2 at different concentrations, specifically 1 μM, there was no correlation between the signal and the concentration of OTA. For switching peptide *H*2, in the same OTA concentration range, there was no correlation between the signal and the concentration, with respect to the formation of the antibody complex, in this immunoassay. These results showed that the one-step OTA immunoassay is feasible when using 2 μM switching peptide *L*2, and the binding site of OTA for the antibody was found to be the binding site of switching peptide *L*2.

The selectivity test of a one-step immunoassay with switching peptides was carried out using phenylalanine and zearalenone. Phenylalanine was reported to be used for the selectivity test of OTA detection because of the similar chemical structure to OTA (Baudrimont et al. 1997) and zearalenone was also used for the selectivity test of OTA because this chemical was a kind of mycotoxin (Lv et al. 2014). As the first step, the selectivity of anti-OTA antibody was estimated using SPR biosensor. As shown in Fig. 3c, the SPR measurement showed that the anti-OTA antibody could discriminate OTA from these two chemicals in the detection range of 500–2500 nM. And then, the selectivity test of a one-step immunoassay based on switching peptide *L*2 was carried out for OTA detection using these two chemicals. As shown in Fig. 3d, the test results showed that the one-step immunoassay with switching peptide was estimated to have a far higher sensitivity for OTA in comparison to these two chemicals. These results showed that the one-step immunoassay with switching peptides could be applied to the detection of OTA for mixtures with similar structures to OTA.

Table 2 Affinity constant (K_D) of OTA and switching peptides (*L*1, *L*2, *H*1, *H*2) against anti-OTA antibody

	OTA	*L*1	*L*2	*H*1	*H*2
Binding constant (K_D)	810 nM	25 μM	12 μM	39 μM	71 μM

Application to OTA analysis in wines

The one-step immunoassay based on switching peptide *L*2 was applied to analyze OTA content in wines. Usually, the analysis of ingredients in foods is carried out according to the directives of related authorities (Schrenk et al. 2020), and an official protocol for OTA analysis within wines has been announced as a directive of the NIH (directive number: 89-2813) (Filali et al. 2001). The

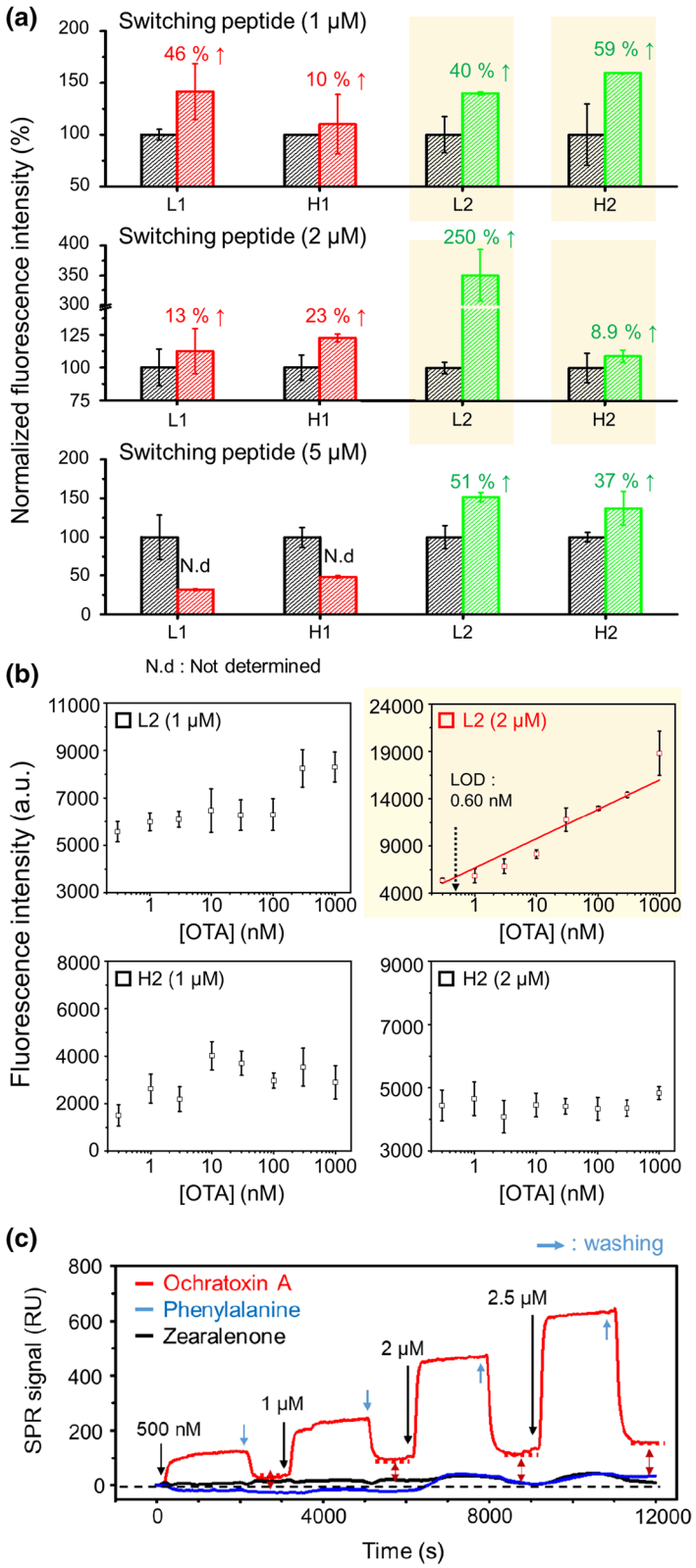

Fig. 3 Releasing test of switching peptides to the Fab region of IgG. **a** Fluorescence responses of four kinds of switching peptides for the detection of OTA. **b** Quantitative one-step immunoassay by the measurement of fluorescence intensity in solution after OTA treatment. **c** SPR sensorgrams of OTA and two comparing chemicals of phenylalanine and zearalenone using immobilized anti-OTA antibodies. **d** Selectivity test of the one-step immunoassay based on switching peptide for OTA using two comparing chemicals of phenylalanine and zearalenone

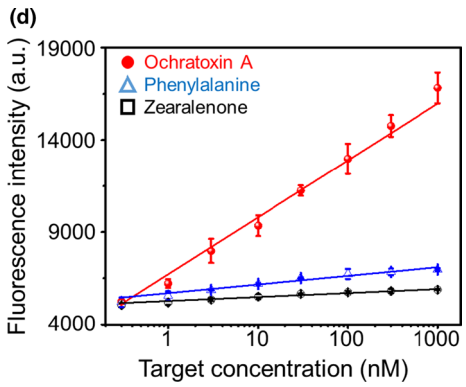

Fig. 3 continued

concentration of OTA in wines is permitted to be less than 2.0 μg/kg, which corresponds to 5 nM. The quantitative analysis of OTA was carried out using HPLC as a reference method. As shown in Fig. 4a, OTA samples in a concentration range of 1 to 30 nM were analyzed, and the peak height was taken as the signal; here, the LOD was estimated to be 2.5 nM.

As the next step, the one-step immunoassay based on the switching peptide was applied for detecting OTA in wines after liquid extraction using chloroform as a solvent. The switching peptide *L*2 was used, and the antibody complex was generated at the optimal concentration of 2.0 μM. The wine samples were prepared by spiking known concentrations of OTA in the range of 1.0 to 1000 nM. As a positive control, OTA samples were prepared in PBS based on the same concentration range. As shown in Fig. 4b fluorescence signal could be measured for the spiked wine samples in the concentration range of 8 to 1000 nM, exhibiting a linear correlation (R^2) of 0.87 ($n=3$). Moreover, the LOD was estimated as 8.5 nM ($n=3$). For the positive control (OTA samples in PBS), a fluorescence signal was detected in the concentration range of 1.0 to 1000 nM ($n=3$), with an LOD of 1.3 nM ($n=3$). These results showed that the liquid extraction using chloroform as a solvent could be effectively used for OTA analysis in wines with a relatively short analysis time (< 1 h) compared to the conventional method.

Wine samples were also directly tested using the one-step immunoassay based on switching peptide. The same assay condition was applied as in the aforementioned experiment, and the wine samples were prepared through dilution with PBS. As shown in Fig. 4c, the fluorescence signal was measured for the spiked wine samples in the concentration range of 1.4 to 3000 nM, with a linear correlation (R^2) of 0.91 ($n=3$). Moreover, the LOD was estimated to be 5.3 nM ($n=3$). For the positive OTA samples in PBS, a fluorescence signal in the concentration range of 1.0 to 1000 nM ($n=3$) was observed, with an LOD of 0.7 nM ($n=3$). These results showed that the sensitivity of OTA analysis increased and that the measurement baseline was increased compared with those of the liquid extraction method. Considering the LOD, the direct dilution method could be effectively used for the one-step immunoassay based on the switching peptide assay to detect OTA in wines with a markedly improved total analysis time (< 0.5 h). The recovery of a one-step immunoassay with switching peptide was carried out using OTA samples spiked in wine. As summarized in Table 3, the OTA concentration was controlled to be 1, 10, 100, 1000 nM ($n=3$), and the recovery was determined from the standard curve. The recovery for real sample measurement was determined to be in the range of 98–109% as summarized in Table 3. These results showed that the one-step immunoassay with switching peptides could be performed with a high recovery rate. Additionally, the assay results of one-step immunoassay based on switching peptides were compared with the lateral flow immunoassay. As shown in Fig. 4d, the detection range of the lateral flow immunoassay was estimated to be 0–50 nM and the LOD was estimated to be 7.8 nM. These results showed that the one-step immunoassay based on switching peptide could have a lower LOD (5.3 nM) as well as a wider detection range (1–3000 nM) in comparison with the lateral flow immunoassay.

Conclusion

A one-step immunoassay based on switching peptides was carried out using an antibody complex with switching peptides. As the first step, the K_D of OTA to the antibodies was estimated by injecting analytes at different concentrations with continuous flow conditions for the SPR biosensor. The K_D of OTA to the antibodies was estimated to be 8.1×10^{-7} M and those of the switching

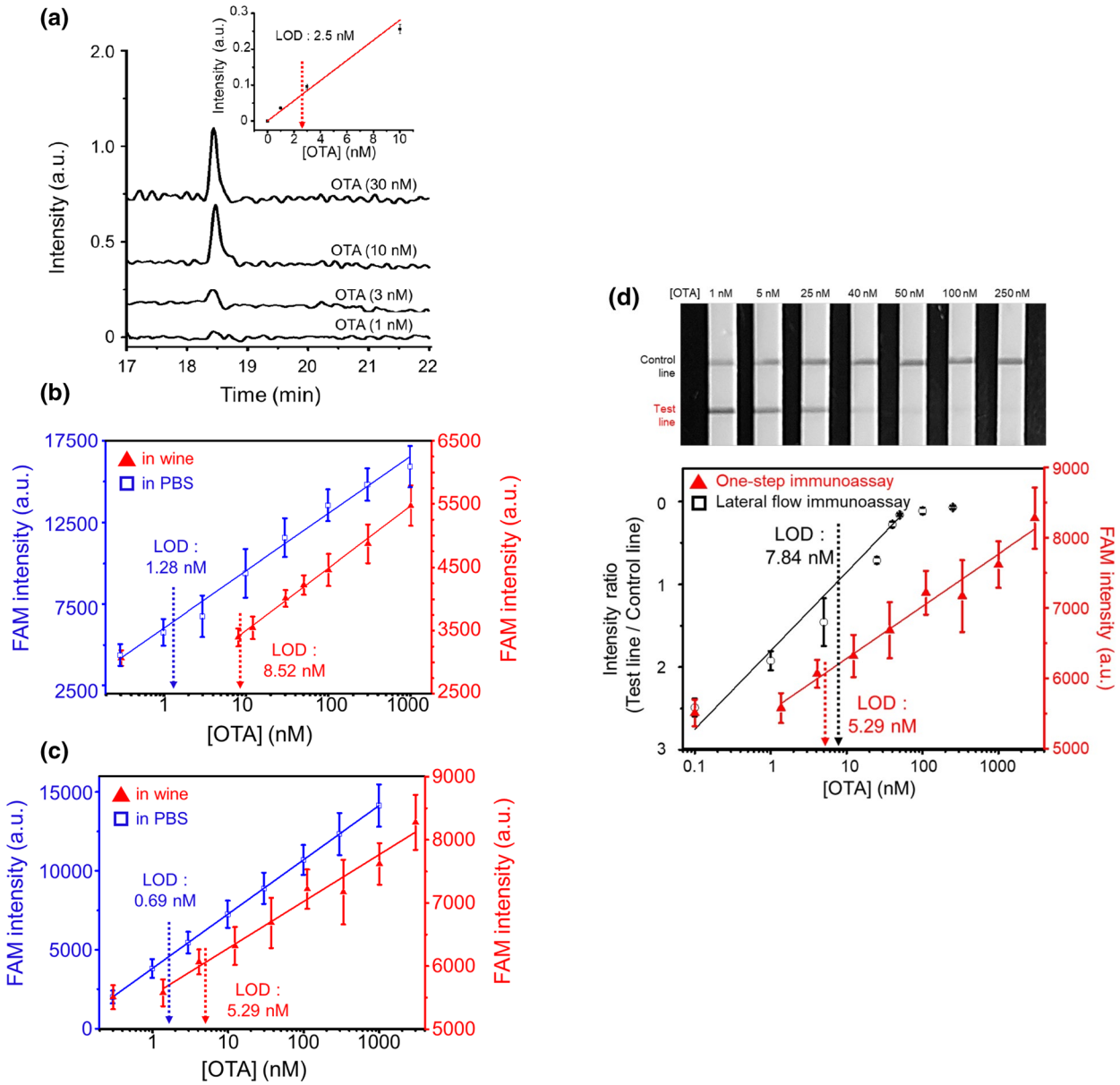

Fig. 4 Quantitative analysis of OTA-spiked wine samples using **a** HPLC, **b** one-step immunoassay based on liquid extraction, **c** one-step immunoassay based on direct dilution, and **d** lateral flow immunoassay

Table 3 Stability of fluorescence signal output to different ratios of wine samples

Spiked OTA (nM)	Detected OTA (nM)	Recovery (%)	RSD (%) ($n=3$)
10	10.9	108.5	15.4
100	107.2	107.2	17.5
1000	982.2	98.2	9.2

*Δ Signal represents the difference of measured signal at the corresponding OTA concentration from the standard curve

peptides were estimated to be in the range of 10^{-5} to 10^{-6} M. From these results, the binding properties of the target analyte (OTA) were determined to be suitable for the one-step immunoassay based on switching peptides. The optimal switching peptide should have a high dissociation ratio compared with that of PBS (negative control). Among four types of switching peptides, *L*2 had the maximum dissociation ratio, and the one-step immunoassay based on switching peptide *L*2 showed a linear correlation ($R^2=0.976$, $n=3$) for OTA in the concentration range of 1 to 1000 nM, with an LOD estimated at

0.60 nM ($n=3$). From the selectivity test using phenylalanine and zearalenone as two comparing chemicals, the one-step immunoassay with switching peptide was estimated to have a far higher sensitivity for OTA in comparison to these two chemicals. For application to the one-step immunoassay for OTA based on switching peptides, two types of sample pre-treatment methods were used. When a liquid extraction was applied using chloroform as a solvent, the fluorescence signal could be measured for the spiked wine samples in the concentration range of 8 to 1000 nM, with a linear correlation (R^2) of 0.869 ($n=3$). Moreover, the LOD was estimated to be 8.5 nM ($n=3$). With the direct dilution of the wine sample, the fluorescence signal could be measured for the spiked wine samples in the concentration range of 1.4 to 3000 nM, with a linear correlation (R^2) of 0.910 ($n=3$). Here, the LOD was estimated to be 5.3 nM ($n=3$). These results showed that the direct wine dilution method can be effectively used for the one-step immunoassay based on a switching peptide assay, to detect OTA in wines with a markedly improved total analysis time (< 0.5 h). In comparison with the lateral flow immunoassay, the one-step immunoassay based on switching peptide could have a lower LOD (5.3 nM) as well as a wider detection range (1–3000 nM).

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40543-022-00352-3.

Additional file 1: Fig. S1. Fluorescence spectra for **a** TAMRA ($\lambda_{ex} = 552$ nm, $\lambda_{ex} = 578$ nm) and **b** FAM ($\lambda_{ex} = 488$ nm, $\lambda_{ex} = 512$ nm).

Acknowledgements
Not applicable.

Author contributions
T-HK contributed to experiment, data curation, and writing—original draft. J-HB and H-RK contributed to experiment and data curation. W-BS supervised the study. M-JK contributed to supervision, review and editing. J-CP contributed to supervision, funding acquisition, writing—original draft, review and editing. All authors read and approved the final manuscript.

Funding
This work is supported by the National Research Foundation of Korea [Grant numbers: NRF-2020R1A2B5B01002187, NRF-2020R1A5A101913111, and NRF-2021R1A2C209370611].

Availability of data materials
No supporting data are included.

Declarations

Competing interests
No competing interests related to this paper.

Author details
[1]Department of Materials Science and Engineering, Yonsei University, 50 Yonsei-Ro, Seodaemun-Gu, Seoul 03722, Republic of Korea. [2]Department of Food Science and Technology, Institute of Agriculture and Life Science, Gyeongsang National University, Jinju, Gyeongnam 52828, Republic of Korea. [3]Korea Institute of Science and Technology (KIST), 5 Hwarang-Ro 14-Gil, Seongbuk-Gu, Seoul 02792, Republic of Korea.

Received: 9 September 2022 Accepted: 3 November 2022
Published online: 14 November 2022

References

Anfossi L, et al. Increased sensitivity of lateral flow immunoassay for ochratoxin A through silver enhancement. Anal Bioanal Chem. 2013;405(30):9859–67.
Aresta A, et al. Ochratoxin A determination in beer by solid-phase microextraction coupled to liquid chromatography with fluorescence detection: a fast and sensitive method for assessment of noncompliance to legal limits. J Agric Food Chem. 2006;54(5):1594–8.
Baudrimont I, Ahouandjivo R, Creppy E. Prevention of lipid peroxidation induced by ochratoxin A in Vero cells in culture by several agents. Chem Biol Interact. 1997;104(1):29–40.
Bellver Soto J, et al. Presence of ochratoxin A (OTA) mycotoxin in alcoholic drinks from southern European countries: wine and beer. J Agric Food Chem. 2014;62(31):7643–51.
Bong J-H, et al. Pig sera-derived anti-SARS-CoV-2 antibodies in surface plasmon resonance biosensors. BioChip J. 2020;14(4):358–68.
Bong J-H, et al. Switching-peptides for one-step immunoassay and its application to the diagnosis of human hepatitis B. Biosens Bioelectron. 2021;178: 112996.
Bui-Klimke TR, Wu F. Ochratoxin A and human health risk: a review of the evidence. Crit Rev Food Sci Nutr. 2015;55(13):1860–9.
Dai S, et al. A luminescence resonance energy transfer based aptasensor for the mycotoxin Ochratoxin A using upconversion nanoparticles and gold nanorods. Microchim Acta. 2016;183(6):1909–16.
De Jesus CL, et al. High incidence and levels of ochratoxin A in wines sourced from the United States. Toxins. 2017;10(1):1.
Filali A, et al. Ochratoxin A in beverages from Morocco: a preliminary survey. Food Addit Contam. 2001;18(6):565–8.
Gupta PK, et al. Amino acid functionalized ZrO 2 nanoparticles decorated reduced graphene oxide based immunosensor. J Mater Chem B. 2017;5(10):2019–33.
Jung J, et al. Anti-SARS-CoV-2 nucleoprotein antibodies derived from pig serum with a controlled specificity. BioChip J. 2021a;15(2):195–203.
Jung J, et al. Isolation of antibodies against the spike protein of SARS-CoV from pig serum for competitive immunoassay. BioChip J. 2021b;15(4):396–405.
Kaarj K, et al. Simple paper-based liver cell model for drug screening. BioChip J. 2020;14(2):218–29.
Karczmarczyk A, et al. Fast and sensitive detection of ochratoxin A in red wine by nanoparticle-enhanced SPR. Anal Chim Acta. 2016;937:143–50.
Khan R, Dhayal M. Nanocrystalline bioactive TiO_2-chitosan impedimetric immunosensor for ochratoxin-A. Electrochem Commun. 2008;10(3):492–5.
Kholová A, et al. Determination of ochratoxin A and ochratoxin B in archived Tokaj wines (vintage 1959–2017) using on-line solid phase extraction coupled to liquid chromatography. Toxins. 2020;12(12):739.
Kim H-R, et al. One-step homogeneous immunoassay for the detection of influenza virus using switching peptide and graphene quencher. BioChip J. 2022a;16:1–8.
Kim H-R, et al. Homogeneous one-step immunoassay based on switching peptides for detection of the influenza virus. Anal Chem. 2022b;94:9627–35.
Liu Y, et al. Molecularly imprinted polymer with mixed-mode mechanism for selective extraction and on-line detection of ochratoxin A in beer sample. Microchem J. 2021;170: 106696.
Lv Z, et al. A simple and sensitive approach for ochratoxin A detection using a label-free fluorescent aptasensor. PLoS ONE. 2014;9(1): e85968.
Lv X, et al. Aptamer-based fluorescent detection of ochratoxin A by quenching of gold nanoparticles. RSC Adv. 2017;7(27):16290–4.
Mateo R, et al. An overview of ochratoxin A in beer and wine. Int J Food Microbiol. 2007;119(1–2):79–83.

Ortiz-Villeda B, et al. Ochratoxins in wines: a review of their occurrence in the last decade, toxicity, and exposure risk in humans. Toxins. 2021;13(7):478.
Park J-H, et al. Electrochemical one-step immunoassay based on switching peptides and pyrolyzed carbon electrodes. ACS Sens. 2022;7(1):215–24.
Pena A, et al. Determination of ochratoxin A in Portuguese rice samples by high performance liquid chromatography with fluorescence detection. Anal Bioanal Chem. 2005;382(5):1288–93.
Rong Z, et al. Dual-color magnetic-quantum dot nanobeads as versatile fluorescent probes in test strip for simultaneous point-of-care detection of free and complexed prostate-specific antigen. Biosens Bioelectron. 2019;145: 111719.
Schrenk D, et al. Risk assessment of ochratoxin A in food. EFSA J. 2020;18(5): e06113.
Studier FW, Moffatt BA. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. J Mol Biol. 1986;189(1):113–30.
Sung JS, et al. One-step immunoassay for food allergens based on screened mimotopes from autodisplayed FV-antibody library. Biosens Bioelectron. 2022;202: 113976.
Taradolsirithitikul P, Sirisomboon P, Dachoupakan Sirisomboon C. Qualitative and quantitative analysis of ochratoxin A contamination in green coffee beans using Fourier transform near infrared spectroscopy. J Sci Food Agric. 2017;97(4):1260–6.
Tessini C, et al. Alternatives for sample pre-treatment and HPLC determination of ochratoxin A in red wine using fluorescence detection. Anal Chim Acta. 2010;660(1–2):119–26.
Ueda T, et al. Improving the soluble expression and purification of recombinant human stem cell factor (SCF) in endotoxin-free *Escherichia coli* by disulfide shuffling with persulfide. Protein Expr Purif. 2016;120:99–105.
Visconti A, Pascale M, Centonze G. Determination of ochratoxin A in wine and beer by immunoaffinity column cleanup and liquid chromatographic analysis with fluorometric detection: collaborative study. J AOAC Int. 2001;84(6):1818–27.
Yoo G, et al. Immobilization of *E. coli* with autodisplayed Z-domains to a surface-modified microplate for immunoassay. Anal Chim Acta. 2011;707(1–2):142–7.
Zou X, et al. Phage-free peptide ELISA for ochratoxin A detection based on biotinylated mimotope as a competing antigen. Talanta. 2016;146:394–400.

Publisher's Note