Food Chemistry 215 (2017) 377–382

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

A simple aptamer-based fluorescent assay for the detection of Aflatoxin B_1 in infant rice cereal

Lu Chen [a,b,c,1], Fang Wen [a,c,1], Ming Li [a,c,1], Xiaodong Guo [a,c], Songli Li [a,c], Nan Zheng [a,c,d,*], Jiaqi Wang [a,c,d,*]

[a] Ministry of Agriculture Laboratory of Quality & Safety Risk Assessment for Dairy Products (Beijing), Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, PR China
[b] College of Animal Science, Xinjiang Agricultural University, Urumchi 830052, PR China
[c] Ministry of Agriculture – Milk and Dairy Product Inspection Center (Beijing), Beijing 100193, PR China
[d] State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, PR China

ARTICLE INFO

Article history:
Received 18 December 2015
Received in revised form 20 May 2016
Accepted 27 July 2016
Available online 2 August 2016

Keywords:
Aflatoxin B_1
Aptamer
Fluorescent
Quenching
Infant rice cereal

ABSTRACT

A fluorescent assay for the rapid, sensitive and specific detection of Aflatoxin B_1 (AFB_1) was developed in this study. Initially, a DNA/DNA duplex was formed between a fluorescein-labeled AFB_1 aptamer and its partially complementary DNA strand containing a quencher moiety, resulting in fluorescence quenching due to the close proximity of fluorophore and quencher. Upon the addition of AFB_1, an aptamer/AFB_1 complex was generated to release the quencher-modified DNA strand, thus recovered the fluorescence of fluorescein and enabled quantitative detection for AFB_1 by monitoring fluorescence enhancement. Under optimized conditions, this assay exhibited a linear response to AFB_1 in the range of 5–100 ng/mL with a detection limit down to 1.6 ng/mL. Trials of this assay in infant rice cereal with satisfactory recovery in the range of 93.0%–106.8%, demonstrate that the new assay could be a potential sensing platform for AFB_1 determination in food.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Mycotoxins are the toxic secondary metabolites produced by a variety of molds, which could cause serious problems to human health through contamination of various foods and animal feeds (Atar, Eren, & Yola, 2015; Mata et al., 2015; Zhu et al., 2015). In recent years, aflatoxins, as the most predominant and toxic mycotoxins, have raised global concern by being frequently detected in agricultural products and causing subsequent economic losses (Pietri, Fortunati, Mulazzi, & Bertuzzi, 2016; Zhang et al., 2016). Among several types of aflatoxins (B_1, B_2, G_1, G_2, M_1, and M_2), Aflatoxin B_1 is known as the most potent carcinogen, and has been classified as a group 1 carcinogen by the International Agency for Research on Cancer (Lee, Her, & Lee, 2015). Some agriculture products, such as peanuts, corn, and cereals, are especially vulnerable to contamination by AFB_1 (Chen et al., 2014; Iqbal, Rabbani, Asi, & Jinap, 2014; Zhang et al., 2016). Therefore, the European Commission has regulated a maximum contamination level for AFB_1 (2 μg/kg) and total aflatoxin (4 μg/kg) in all cereals and cereal-derived products to protect consumers' health and prevent food safety issues (Commission, 2010). Given of the low permissible limit and severe toxicity of AFB_1, rapid, sensitive and specific analytical methods for AFB_1 determination are vitally important.

Analytical methods based on thin layer chromatography (TLC) (Var, Kabak, & Gök, 2007), high-performance liquid chromatography (HPLC) (Herzallah, 2009; Yazdanpanah et al., 2013), and liquid chromatography combined with mass spectrometry (LC–MS) (Abia et al., 2013; Warth, Sulyok, & Krska, 2013) have been developed for the detection of AFB_1. However, these typical instrumental methods usually require special equipments and professional personnel, as well as time-consuming sample pretreatment (Shim et al., 2007). Meanwhile, rapid detection methods based on immunoassays have been widely used for AFB_1 measurements in food and agricultural products (Mozaffari Nejad, Sabouri Ghannad, & Kamkar, 2014; Sheng et al., 2014; Xu et al., 2014). These antibody-based detection methods, while highly selective, are often limited by high cost and easy-to-denature during storage, making them difficult for real-time and on-site detection (Huang, Zhao, Chen, Shi, & Liang, 2012). As an alternative method to antibody for small molecules recognition, aptamer is a single-stranded DNA or RNA oligonucleotides, which could bind to a target with very high affinity and specificity. With the advantages of less

* Corresponding authors at: Ministry of Agriculture Laboratory of Quality & Safety Risk Assessment for Dairy Products (Beijing), Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, PR China.
E-mail addresses: zhengnan_1980@126.com (N. Zheng), jiaqiwang@vip.163.com (J. Wang).
[1] The authors contributed equally.

http://dx.doi.org/10.1016/j.foodchem.2016.07.148
0308-8146/© 2016 Elsevier Ltd. All rights reserved.

expensive, facile synthesis, ease modification, and high stability under non-physiological conditions (Jayasena, 1999), aptamer-based biosensors have been developed for the detection of mycotoxin, such as ochratoxin A (OTA) and fumonisin B_1 (FB_1) (Z. Guo, Ren, Wang, & Wang, 2011; Kuang et al., 2010; Wu et al., 2012, 2013; Yang, Lates, Prieto-Simón, Marty, & Yang, 2013). In 2012, specific aptamers to AFB_1 had been patented by Neoventures Biotechnology Inc. (Canada) (Patent: PCT/CA2010/001292), after which studies on aptasensor for AFB_1 determination have emerged and successfully applied to agricultural products (Castillo et al., 2015; Evtugyn et al., 2014; Seok, Byun, Shim, & Kim, 2015; Shim, Kim, Mun, & Kim, 2014; Wang et al., 2016). For example, an aptamer-based dipstick assay based on a competitive reaction of the biotinylated form of aptamer and cy5-modified DNA probes has been recently developed for detection of AFB_1, achieving a LOD of 0.32 nM (Shim, Kim, et al., 2014). Fluorescent nitrogen-doped carbon dots (N,C-dots) were assembled on aptamer modified gold nanoparticles (Aptamer/AuNPs) for sensitive detection of AFB_1 (Wang et al., 2016). Previously, our group reported an ultrasensitive aptasensor based on configurational changes of the aptamer specific to AFB_1 immobilized in PCR tubes (Guo et al., 2014). However, the reported aptamer assays for AFB_1 based on real-time quantitative polymerase chain reaction (RT-qPCR) are time-consuming, either require complicated sample preparation or long incubation time in rigorous conditions. Therefore, simple, rapid and low-cost methods for quantifying AFB_1 levels in food and animal feeds are still challenging.

In this study, we report a novel fluorescent assay for the detection of AFB_1 using a fluorophore-labeled AFB_1 aptamer and its partially complementary DNA strand (denoted as cDNA) that is covalently modified with a quencher. In the absence of AFB_1, the aptamer naturally binds to cDNA, bringing the fluorophore and quencher into close proximity to induce highly efficient fluorescence quenching. After introducing AFB_1, the aptamer prefers to form an aptamer/AFB_1 complex rather than the DNA/DNA duplex, triggering the release of cDNA, which is accompanied by the increase of fluorescence. The whole sensing procedures are simple, rapid, and shows high specificity to AFB_1, which offers great potential for on-site analysis. Measurement of AFB_1 levels in spiked infant rice cereal samples is also achieved, demonstrating the practicability of this approach.

2. Experimental

2.1. Materials and reagents

Aflatoxin B_1 (AFB_1), Ochratoxin A (OTA), Aflatoxin M_1 (AFM_1), Aflatoxin B_2 (AFB_2), Aflatoxin G_1 (AFG_1), Aflatoxin G_2 (AFG_2), Zearalenone (ZEA) and Fumonisin B_1 (FB_1) were purchased from the National Standard Reference Center (Beijing, China). Other chemical reagents such as sodium chloride (NaCl), potassium chloride (KCl), 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris), and anhydrous calcium chloride ($CaCl_2$) were purchased from Shanghai Chemical Reagent Company (Shanghai, China). Infant rice cereal samples (Nestle and Heinz) were purchased from local supermarket. Water was purified using a Milli-Q purification system. Tris-HCl buffer (10 mM Tris, 120 mM NaCl, 5 mM KCl, 20 mM $CaCl_2$, at pH 7.0) was used for fluorescent assay.

The oligonucleotides used in this work were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China) and purified with HPLC. The sequence of AFB_1 aptamer was according to Patent: PCT/CA2010/001292 with modification (Guo et al., 2014). The sequences were as follows:

AFB_1 Aptamer: 5′-GT TGG GCA CGT GTT GTC TCT CTG TGT CTC GTG CCC TTC GCT AGG CCC-FAM-3′

Partially Complementary DNA (cDNA):cDNA1: 5′-TAMRA-GGG CCT AG-3′cDNA2: 5′-TAMRA-GGG CCT AGC-3′cDNA3: 5′-TAMRA-GGG CCT AGC G-3′cDNA4: 5′-TAMRA-GGG CCT AGC GA-3′cDNA5: 5′-TAMRA-GGG CCT AGC GAA-3′

2.2. Apatmer-based fluorescent assay for AFB_1 detection

AFB_1 aptamer and cDNA were dissolved and diluted with Tris-HCl buffer. Then, 500 μL of AFB_1 aptamer solution (10 nM) was mixed with 500 μL of cDNA at molar ratios of 1:1, 1:2, 1:3, 1:4 and 1:5 (unless stated otherwise, a molar ratio of 1:2 was used for AFB_1 analysis). The mixture was heated to 88 °C for 5 min and incubated at room temperature for 30 min. Then, 500 μL of AFB_1 standard solution of different concentrations was added to the mixture, giving a final reaction volume of 1.5 mL. After votexing, the fluorescence intensity at excitation/emission wavelength of 495/520 nm was recorded by F-7000 fluorophotometer (Hitachi, Japan). Each concentration was measured for five times. Considering aflatoxin is a highly fluorescent substance (Ex/Em: 360/420 nm for AFB_1), the background fluorescence signal of AFB_1 might overlap with the fluorescence signal of FAM modified aptamer. To avoid the influence of background fluorescence signal of aflatoxins, we used background correction method by measuring the fluorescence of the control, which contains AFB_1 and the buffer, but without FAM modified AFB_1 aptamer and cDNA.

2.3. Specificity analysis

To prove the ability of this method for highly selective detection of AFB_1 over other mycotoxins, 40 ng/mL of OTA, ZEA, FB_1, AFM_1, AFB_2, AFG_1 and AFG_2 were measured at the same conditions as AFB_1.

2.4. Analysis of AFB_1 spiked infant rice cereal samples

The practicability of this assay was verified by quantitative detection of AFB_1 in two brands of infant rice cereal samples that were spiked with known concentration of AFB_1. Each sample was spiked with AFB_1 at concentrations of 5, 10, 20 μg/kg, respectively. Each sample was accurately weighed (2.00 ± 0.05 g) and 10 mL of extraction solution (methanol: water, 6:4) was then added to extract AFB_1 from the sample. The entire mixture was vortexed for 5 min using a Vortex-Genie 2 (Scientific Industries, USA) and then centrifuged at 10,000*g* for 10 min. The supernatant was collected, filtered through a 0.45 μm filter, and diluted with Tris buffer (1:5, v:v) for recovery experiments. Five repeats were measured for each sample to assess the accuracy of the assay.

3. Results and discussion

3.1. Design of the aptamer-based fluorescent sensing platform

Schematic illustration for the design of fluorescent assay for AFB_1 detection was displayed in Fig. 1A. In this sensing system, a AFB_1 aptamer was labeled with Carboxyfluorescein (FAM) and its partially complementary DNA was modified with Carboxytetramethylrhodamine (TAMRA) quenching group. Without introducing AFB_1, the hybridization of AFB_1 aptamer and cDNA resulted in a close proximity for FAM and TAMRA quenching group. In this case, the fluorescence of FAM was effectively quenchend. Upon the addition of AFB_1, structural switch of AFB_1 aptamer was induced, leading to the formation of AFB_1/aptamer complex (Seok et al., 2015). Thereby, the cDNA was dehybridized from AFB_1 aptamer and recovered the fluorescence of FAM. To confirm that the presence of AFB_1 could result in dehybridization of aptamer/cDNA

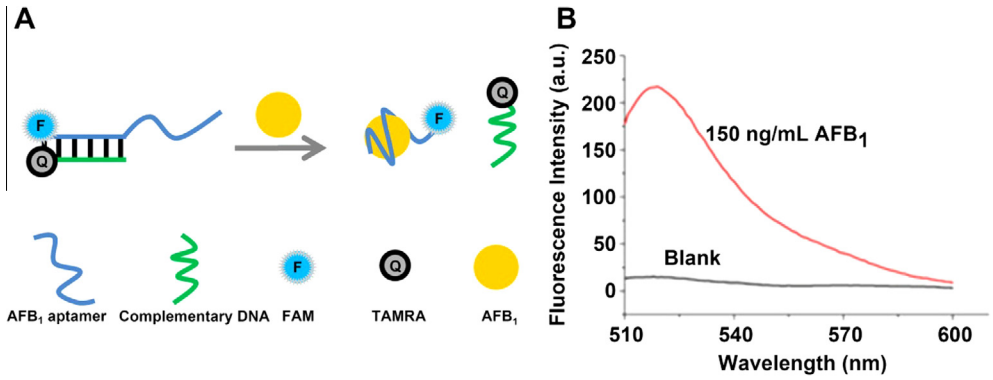


Fig. 1. (A) Schematic illustration of the aptasensor for the detection of AFB_1. (B) Fluorescence emission spectra of the aptamer-based sensing system in the absence (blank) and presence of 150 ng/mL AFB_1. Excitation and emission are at $\lambda_{ex}/\lambda_{em}$ = 495/520 nm. Conditions: 10 nM AFB_1 aptamer, 20 nM cDNA in Tris-HCl buffer (10 mM Tris, 120 mM NaCl, 5 mM KCl, 20 mM $CaCl_2$, at pH 7.0).

duplex and subsequent fluorescence enhancement, 150 ng/mL of AFB_1 was added to a Tris-HCl buffer solution containing 10 nM AFB_1 aptamer and 20 nM cDNA. As shown in Fig. 1B, a more than 14-fold fluorescence enhancement was observed after 15 min, suggesting that the AFB_1/aptamer complex was formed. Moreover, it means that the covalently labeled fluorophore (FAM) did not perturb the original recognition property of AFB_1 aptamer.

3.2. Optimization of the sequence for cDNA

The optimization for the sequence of cDNA is essential for both enhancing the stability of aptamer/cDNA duplex to achieve low background fluorescence in the absence of AFB_1, and facilitating the target-induced dehybridization of AFB_1 aptamer and cDNA upon the addition of AFB_1 for higher fluorescence enhancement. Therefore, a series of cDNA with various length were designed to optimize the sensing performance of the fluorescence assay. As shown in Fig. 2A, blue columns showed the fluorescence intensity of samples without AFB_1, while purple columns represented that of the samples in the presence of 150 ng/mL of AFB_1. The results showed that cDNA2, cDNA3, cDNA4 and cDNA5 could all effectively eliminate the fluorescence of FAM and reduce the background signal in the absence of AFB_1. However, cDNA1 made high background fluorescence owing to its short length, which would weaken the hybridization with AFB_1 aptamer. Upon the addition of AFB_1, a significant fluorescence enhancement (F/F_0) was observed when cDNA2 was applied as the partially complementary DNA, while longer cDNA sequences (cDNA3, cDNA4 and cDNA5) resulted in lower fluorescence enhancement (Fig. 2B). These might because that cDNA with more nucleotides could facilitate the formation of cDNA/aptamer duplex, which limited the target-induced structural switch of AFB_1 apatmer. Therefore, cDNA2 with

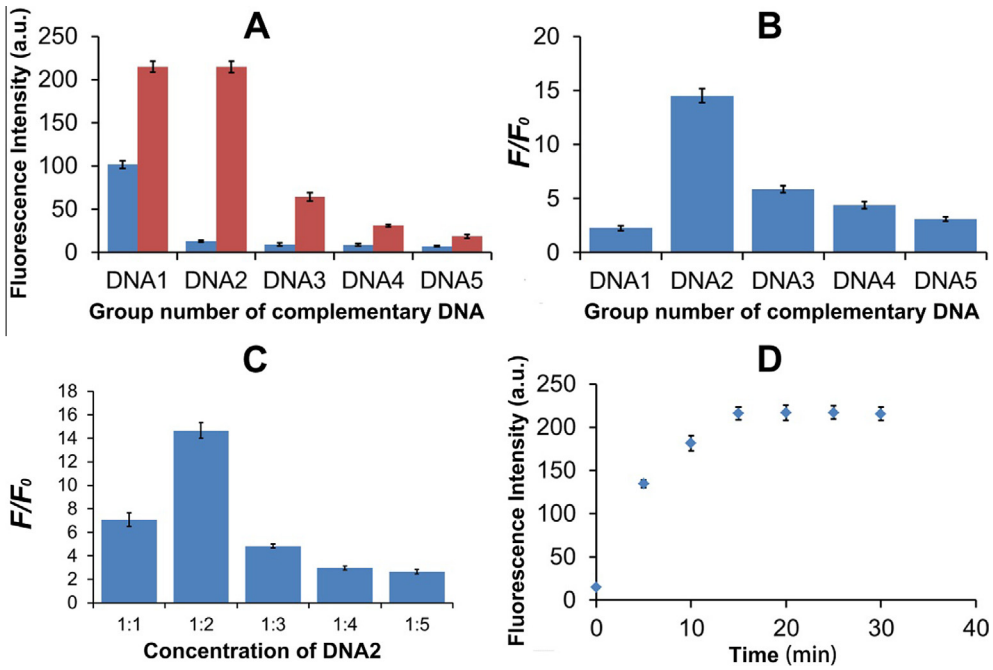


Fig. 2. Optimization of cDNA, cDNA2 and reaction time. (A) Optimization of cDNA with various amount of nucleotides (cDNA1, cDNA2, cDNA3, cDNA4, cDNA5). Blue bars represented the fluorescence intensity before the addition of AFB_1, purple bars represented the samples with 150 ng/mL of AFB_1. (B) Fluorescence enhancement (F/F_0) upon the addition of 150 ng/mL AFB_1. (C) Optimization of the concentration for cDNA2, blue columns represented the fluorescence enhancement (F/F_0) upon the addition of 150 ng/mL AFB_1. (D) Optimization of the reaction time. The concentration of AFB_1 was 150 ng/mL. Every data point was the mean of three measurements. The error bars are the standard deviation. Excitation and emission are at $\lambda_{ex}/\lambda_{em}$ = 495/520 nm. Conditions: 10nM AFB_1 aptamer, 20nM cDNA in Tris-HCl buffer (10 mM Tris, 120 mM NaCl, 5 mM KCl, 20 mM $CaCl_2$, at pH 7.0). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

nine nucleotides complementary to AFB_1 aptamer was used as the optimized sequence.

3.3. Optimization of the concentration for cDNA2

To optimize the sensing performance, the effect of concentration for cDNA2 was also investigated. In this experiment, 150 ng/mL of AFB_1 was added to a solution containing 10 nM AFB_1 aptamer and varies concentrations of cDNA2. As shown in Fig. 2C, a maximum fluorescence enhancement (F/F_0) was reached when the concentration of cDNA2 was 20 nM, suggesting that, even though higher concentration of cDNA2 could provide lower background fluorescence by forming stable cDNA2/aptamer duplex, if the concentration of cDNA2 was higher than 20 nM (2 eqv. of the concentration for AFB_1 aptamer), it would affect the interaction between AFB_1 and its aptamer and hence restrict the increase of fluorescence. In this case, the optimal concentration of cDNA2 was determined as 20 nM for further sensing experiments.

3.4. Optimization the reaction time

To obtain stable assay performance, the influence of reaction time for the target-induced fluorescence enhancement was further investigated. As shown in Fig. 2D, the fluorescence intensity increased dramatically within 15 min after the addition of AFB_1 and reached a plateau at a binding time over 15 min. On this basis, all the fluorescent measurements were initiated after the addition of AFB_1 for 15 min.

3.5. Quantitative determination of AFB_1

Under optimal conditions, the flourescent assay was used for quantitative determination of AFB_1 with excitation and emission wavelengths of 495 nm and 520 nm, respectively. As shown in Fig. 3A, the fluorescence intensity increased with increasing concentration of AFB_1 and reached to a maximum fluorescence enhancement when the concentration of AFB_1 was up to 150 ng/mL. The calibration curve of fluorescence intensity versus AFB_1 concentrations was linear over the range of 5–100 ng/mL (Fig. 3B) as $F = 1.384\ C + 24.964$ ($R^2 = 0.9979$), in which F was the fluorescence intensity and C was the concentration of AFB_1. The detection limit of this method was calculated by $3\sigma_b$/slope (σ_b, standard deviation of the blank samples) to be 1.6 ng/mL. As shown in Table 1, this proposed assay performed comparable sensitivity compared with other instrumental and rapid screening methods.

3.6. Selectivity of the assay

In order to determine the specificity of this method, 40 ng/mL of mycotoxins (OTA, AFB_2, AFM_1, AFG_1, AFG_2, ZEA, FB_1) were measured under the same experiment conditions as AFB_1. As shown in Fig. 4, only AFB_1 induced significant fluorescence increase, while

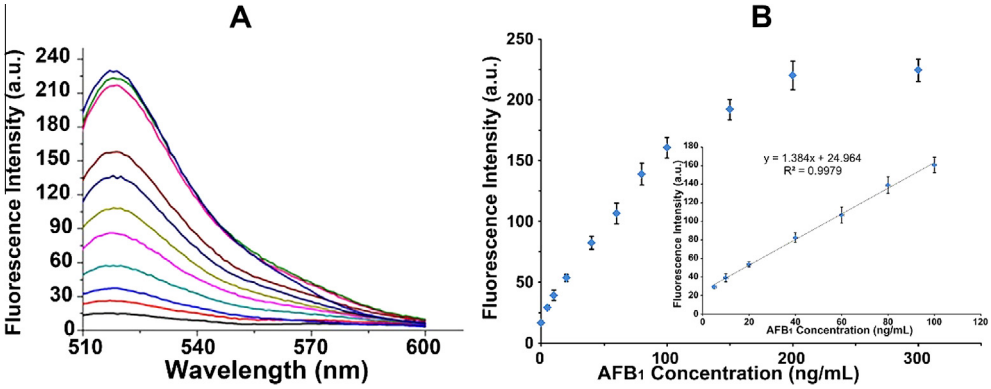


Fig. 3. (A) Fluorescence emission spectra of the aptasensor in the addition of AFB_1 at various concentrations (from bottom to top): 0, 5, 10, 20, 40, 60, 80, 100, 150, 200, 300 ng/mL. (B) Linear relationship between the fluorescence intensity and AFB_1 concentration. Every data point was the mean of three measurements. The error bars are the standard deviation. Excitation and emission are at $\lambda_{ex}/\lambda_{em}$ = 495/520 nm. Conditions: 10 nM AFB_1 aptamer, 20 nM cDNA in Tris-HCl buffer (10 mM Tris, 120 mM NaCl, 5 mM KCl, 20 mM $CaCl_2$, at pH 7.0).

Table 1
Comparison of the sensitivity of currently available methods for the detection of AFB_1.

Method		LOD	References
Instrumental Methods	UHPLC-FLD	2 ng/mL (6.4 nM)	Corcuera, Ibanez-Vea, Vettorazzi, Gonzalez-Penas, and Cerain (2011)
Antibody based methods	Immunochromatography assay	2.5 ng/mL (8 nM)	Xiulan, Xiaolian, Jian, Zhou, and Chu (2005)
	Clean-up tandem immunoassay column	5 ng/mL (16 nM)	Goryacheva et al. (2007)
	Enzyme immunoassay	2 ng/mL (6.4 nM)	Saha, Acharya, Roy, Shrestha, and Dhar (2007)
Aptamer based methods	RT-qPCR	25 fg/mL (80 fM)	Guo et al. (2014)
	Electrochemical Aptasensor based on electropolymerized Neutral red	0.016 ng/mL (0.05 nM)	Evtugyn et al. (2014)
	Electrochemical aptasensor based on Cys-PAMAM dendrimers	0.12 ng/mL (0.4 nM)	Castillo et al. (2015)
	Aptamer-based dipstick assay	0.1 ng/ml (0.32 nM)	Shim, Kim, et al., 2014
	Colorimetric sensor based on aptamer/split DNAzyme	0.1 ng/mL (0.3 nM)	Seok et al. (2015)
	Enzymatic method based on AChE inhibition	10 ng/mL (32 nM)	Moscone, Arduini, and Amine (2011)
	Aptamer-based fluorescent assay	1.6 ng/mL (5.1 nM)	Present work

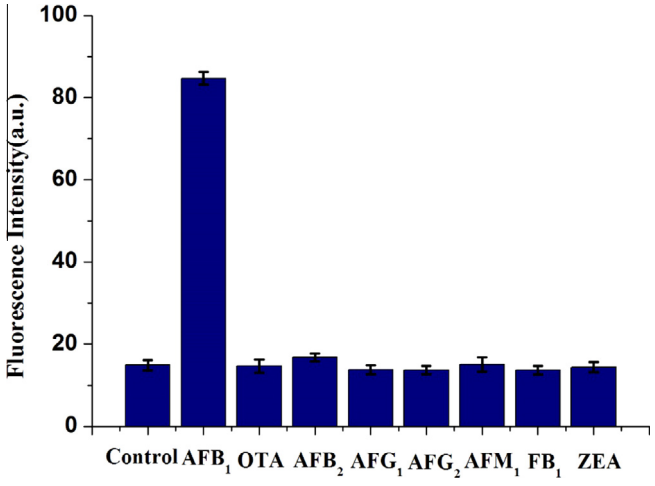


Fig. 4. Fluorescence intensity in the absence (control) and presence of 40 ng/mL mycotoxins: AFB_1, OTA, AFB_2, AFG_1, AFG_2, AFM_1, ZEA, FB_1, The experiment conditions are as following: Excitation and emission are at $\lambda_{ex}/\lambda_{em}$ = 495/520 nm. 10 nM AFB_1 aptamer, 20 nM cDNA2 in Tris-HCl buffer (10 mM Tris, 120 mM NaCl, 5 mM KCl, 20 mM $CaCl_2$, at pH 7.0). Every data point was the mean of three measurements. The error bars are the standard deviation.

no obvious fluorescence intensity changes were observed for other tested mycotoxins. There might be two possible reasons. Firstly, shortening the ligand might change the binding affinity and selectivity, as indicated in Patent (PCT/CA2010/001292). In this study and our previous study (Guo et al., 2014), the shortened AFB_1 aptamer still remained the binding affinity, and good selectivity to AFB_1 against other toxins like AFB_2. Secondly, although both methods used the same aptamer specific to AFB_1, the different results of both methods with cross-reactivity test were reasonable, since the performances and formats of the both assays were different. As discussed in previous report (Shim, Kim et al., 2014), cross-reactivity of analytical methods based on bioreceptors such as aptamers or antibodies could be different even if the methods were developed with the same bioreceptor. The dipstick assay developed by Shim et al. was confirmed to be highly specific to AFB_1, and no cross-reaction to other mycotoxin was observed. Moreover, in their previous report with a chemiluminescence assay based on the same aptamer to AFB_1 modified with DNAzyme and performed on a wells of microtitre plate (Shim et al., 2014), the chemiluminescence assay based on the same aptamer used in that study showed cross-reaction to AFG_1 and zearalenone. The results demonstrated that the aptamer would not react with other mycotoxins except AFB_1, suggesting that this assay had high specificity for the detection of AFB_1.

3.7. Detection of AFB_1 in spiked infant rice cereal samples

To evaluate the practical applicability and reliability of this method, the assay was applied to the detection of AFB_1 in spiked infant rice cereal samples. Two brands of infant rice cereal were selected to spike with AFB_1 at 5, 10, and 20 μg/kg. As shown in Table 2, the recoveries were in the range of 96.3%–106.8% and 93%–101.2%, respectively. These results demonstrated that the assay developed in this study could be useful as a quantitative method for AFB_1 detection in real samples.

Table 2
Determination of AFB_1 spiked infant rice cereal samples (n = 5).[a]

Brand	Added (ng/mL)	Found (ng/mL)	RSD (%)	Recovery (%)
A	5	5.3	2.8	106.8
	10	9.8	3.5	98.4
	20	19.3	3.2	96.3
B	5	5.1	5	101.2
	10	9.3	2.9	93.0
	20	19.2	2.2	95.8

[a] The mean of five experiment.

4. Conclusions

In this study, an aptamer-based fluorescent assay has been successfully developed for simple, rapid, and selective detection of AFB_1. Upon the addition of AFB_1, the formation of AFB_1/aptamer complex induces the dehybridization of fluorophor-labeled aptamer and quencher-labeled cDNA, which provides a sensitive signal-on sensing platform for AFB_1 detection. Under optimal conditions, the fluorescence intensity increase linearly with AFB_1 concentration over the range of 5–100 ng/mL, and a detection limit of 1.6 ng/mL was obtained. This assay also showed excellent selectivity for AFB_1 against other tested mycotoxins (OTA, AFB_2, AFM_1, AFG_1, AFG_2, ZEA, FB_1). Satisfactory recovery were achieved in the measurement of infant rice cereal samples, which were spiked with different concentrations of AFB_1, With the advantages of simple design, low cost, high sensitivity and selectivity, and rapid detection, the proposed method could be provided as a useful tool for on-site analysis of AFB_1 in food samples.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (No. 21305158), Special Fund for Agro-scientific Research in the Public Interest (201403071), Project of Risk Assessment on Raw Milk (GJFP2016008), Modern Agro-Industry Technology Research System of the PR China (nycytx-04-01), and The Agricultural Science and Technology Innovation Program (CARS-37).

References

Abia, W. A., Warth, B., Sulyok, M., Krska, R., Tchana, A. N., Njobeh, P. B., ... Moundipa, P. F. (2013). Determination of multi-mycotoxin occurrence in cereals, nuts and their products in Cameroon by liquid chromatography tandem mass spectrometry (LC-MS/MS). *Food Control, 31*(2), 438–453.

Atar, N., Eren, T., & Yola, M. L. (2015). A molecular imprinted SPR biosensor for sensitive determination of citrinin in red yeast rice. *Food Chemistry, 184*, 7–11.

Castillo, G., Spinella, K., Poturnayová, A., Šnejdárková, M., Mosiello, L., & Hianik, T. (2015). Detection of aflatoxin B1 by aptamer-based biosensor using PAMAM dendrimers as immobilization platform. *Food Control, 52*, 9–18.

Chen, R., Ma, F., Li, P. W., Zhang, W., Ding, X. X., Zhang, Q., ... Xu, B. C. (2014). Effect of ozone on aflatoxins detoxification and nutritional quality of peanuts. *Food Chemistry, 146*, 284–288.

Commission, T. E. (2010). Commission Regulation (EU) No 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. *Official Journal of the European Union*. L50/58-L50/12.

Corcuera, L. A., Ibanez-Vea, M., Vettorazzi, A., Gonzalez-Penas, E., & Cerain, A. L. (2011). Validation of a UHPLC-FLD analytical method for the simultaneous quantification of aflatoxin B1 and ochratoxin a in rat plasma, liver and kidney. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences, 879*(26), 2733–2740.

Evtugyn, G., Porfireva, A., Stepanova, V., Sitdikov, R., Stoikov, I., Nikolelis, D., & Hianik, T. (2014). Electrochemical aptasensor based on polycarboxylic macrocycle modified with neutral red for aflatoxin B1 detection. *Electroanalysis, 26*(10), 2100–2109.

Goryacheva, I. Y., De Saeger, S., Delmulle, B., Lobeau, M., Eremin, S. A., Barna-Vetro, I., & Van Peteghem, C. (2007). Simultaneous non-instrumental detection of aflatoxin B1 and ochratoxin A using a clean-up tandem immunoassay column. *Analytica Chimica Acta, 590*(1), 118–124.

Guo, X. D., Wen, F., Zheng, N., Luo, Q. J., Wang, H. W., Wang, H., ... Wang, J. Q. (2014). Development of an ultrasensitive aptasensor for the detection of aflatoxin B-1. *Biosensors & Bioelectronics, 56*, 340–344.

Guo, Z., Ren, J., Wang, J., & Wang, E. (2011). Single-walled carbon nanotubes based quenching of free FAM-aptamer for selective determination of ochratoxin A. *Talanta, 85*(5), 2517–2521.

Herzallah, S. M. (2009). Determination of aflatoxins in eggs, milk, meat and meat products using HPLC fluorescent and UV detectors. *Food Chemistry, 114*(3), 1141–1146.

Huang, Y., Zhao, S., Chen, Z. F., Shi, M., & Liang, H. (2012). Amplified fluorescence polarization aptasensors based on structure-switching-triggered nanoparticles enhancement for bioassays. *Chemical Communications, 48*(60), 7480–7482.

Iqbal, S. Z., Rabbani, T., Asi, M. R., & Jinap, S. (2014). Assessment of aflatoxins, ochratoxin A and zearalenone in breakfast cereals. *Food Chemistry, 157*, 257–262.

Jayasena, S. D. (1999). Aptamers: An emerging class of molecules that rival antibodies in diagnostics. *Clinical Chemistry, 45*(9), 1628–1650.

Kuang, H., Chen, W., Xu, D., Xu, L., Zhu, Y., Liu, L., ... Zhu, S. (2010). Fabricated aptamer-based electrochemical "signal-off" sensor of ochratoxin A. *Biosensors and Bioelectronics, 26*(2), 710–716.

Lee, J., Her, J. Y., & Lee, K. G. (2015). Reduction of aflatoxins (B(1), B(2), G(1), and G(2)) in soybean-based model systems. *Food Chemistry, 189*, 45–51.

Mata, A. T., Ferreira, J. P., Oliveira, B. R., Batoreu, M. C., Barreto Crespo, M. T., Pereira, V. J., & Bronze, M. R. (2015). Bottled water: analysis of mycotoxins by LC-MS/MS. *Food Chemistry, 176*, 455–464.

Moscone, D., Arduini, F., & Amine, A. (2011). A rapid enzymatic method for aflatoxin B detection. *Methods in Molecular Biology, 739*, 217–235.

Mozaffari Nejad, A. S., Sabouri Ghannad, M., & Kamkar, A. (2014). Determination of aflatoxin B1 levels in Iranian and Indian spices by ELISA method. *Toxin Reviews, 33*(4), 151–154.

Pietri, A., Fortunati, P., Mulazzi, A., & Bertuzzi, T. (2016). Enzyme-assisted extraction for the HPLC determination of aflatoxin M1 in cheese. *Food Chemistry, 192*, 235–241.

Saha, D., Acharya, D., Roy, D., Shrestha, D., & Dhar, T. K. (2007). Simultaneous enzyme immunoassay for the screening of aflatoxin B1 and ochratoxin A in chili samples. *Analytica Chimica Acta, 584*(2), 343–349.

Seok, Y., Byun, J. Y., Shim, W. B., & Kim, M. G. (2015). A structure-switchable aptasensor for aflatoxin B1 detection based on assembly of an aptamer/split DNAzyme. *Analytica Chimica Acta, 886*, 182–187.

Sheng, Y. J., Eremin, S., Mi, T. J., Zhang, S. X., Shen, J. Z., & Wang, Z. H. (2014). The development of a fluorescence polarization immunoassay for aflatoxin detection. *Biomedical and Environmental Sciences, 27*(2), 126–129.

Shim, W. B., Kim, M. J., Mun, H., & Kim, M. G. (2014a). An aptamer-based dipstick assay for the rapid and simple detection of aflatoxin B1. *Biosensors & Bioelectronics, 62*, 288–294.

Shim, W. B., Mun, H., Joung, H. A., Ofori, J. A., Chung, D. H., & Kim, M. G. (2014b). Chemiluminescence competitive aptamer assay for the detection of aflatoxin B1 in corn samples. *Food Control, 36*(1), 30–35.

Shim, W. B., Yang, Z. Y., Kim, J. S., Kim, J. Y., Kang, S. J., Woo, G. J., ... Chung, D. H. (2007). Development of immunochromatography strip-test using nanocolloidal gold-antibody probe for the rapid detection of aflatoxin B1 in grain and feed samples. *Journal of Microbiology and Biotechnology, 17*(10), 1629–1637.

Var, I., Kabak, B., & Gök, F. (2007). Survey of aflatoxin B1 in helva, a traditional Turkish food, by TLC. *Food Control, 18*(1), 59–62.

Wang, B., Chen, Y., Wu, Y., Weng, B., Liu, Y., Lu, Z., ... Yu, C. (2016). Aptamer induced assembly of fluorescent nitrogen-doped carbon dots on gold nanoparticles for sensitive detection of AFB1. *Biosensors & Bioelectronics, 78*, 23–30.

Warth, B., Sulyok, M., & Krska, R. (2013). LC-MS/MS-based multibiomarker approaches for the assessment of human exposure to mycotoxins. *Analytical and Bioanalytical Chemistry, 405*(17), 5687–5695.

Wu, S., Duan, N., Li, X., Tan, G., Ma, X., Xia, Y., ... Wang, H. (2013). Homogenous detection of fumonisin B1 with a molecular beacon based on fluorescence resonance energy transfer between NaYF4: Yb, Ho upconversion nanoparticles and gold nanoparticles. *Talanta, 116*, 611–618.

Wu, S., Duan, N., Ma, X., Xia, Y., Wang, H., Wang, Z., & Zhang, Q. (2012). Multiplexed fluorescence resonance energy transfer aptasensor between upconversion nanoparticles and graphene oxide for the simultaneous determination of mycotoxins. *Analytical Chemistry, 84*(14), 6263–6270.

Xiulan, S., Xiaolian, Z., Jian, T., Zhou, J., & Chu, F. S. (2005). Preparation of gold-labeled antibody probe and its use in immunochromatography assay for detection of aflatoxin B1. *International Journal of Food Microbiology, 99*(2), 185–194.

Xu, W., Xiong, Y., Lai, W., Xu, Y., Li, C., & Xie, M. (2014). A homogeneous immunosensor for AFB1 detection based on FRET between different-sized quantum dots. *Biosensors and Bioelectronics, 56*, 144–150.

Yang, C., Lates, V., Prieto-Simón, B., Marty, J.-L., & Yang, X. (2013). Rapid high-throughput analysis of ochratoxin A by the self-assembly of DNAzyme–aptamer conjugates in wine. *Talanta, 116*, 520–526.

Yazdanpanah, H., Zarghi, A., Shafaati, A. R., Foroutan, S. M., Aboul-Fathi, F., Khoddam, A., ... Shaki, F. (2013). Analysis of aflatoxin B1 in Iranian foods using HPLC and a monolithic column and estimation of its dietary intake. *Iranian Journal of Pharmaceutical Research, 12*, 81–87.

Zhang, X., Li, C. R., Wang, W. C., Xue, J., Huang, Y. L., Yang, X. X., ... Qiu, J. F. (2016). A novel electrochemical immunosensor for highly sensitive detection of aflatoxin B1 in corn using single-walled carbon nanotubes/chitosan. *Food Chemistry, 192*, 197–202.

Zhu, R., Feussner, K., Wu, T., Yan, F., Karlovsky, P., & Zheng, X. (2015). Detoxification of mycotoxin patulin by the yeast *Rhodosporidium paludigenum*. *Food Chemistry, 179*, 1–5.