RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Journal Name

ARTICLE

An indirect competitive fluorescence assay for ochratoxin A based on molecular beacon

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Xiaowen Dou[a], Xianfeng Chu[a], Weijun Kong[a], Jiaoyang Luo[a], Meihua Yang[*a, b]

Abstract: A novel, simple and efficient method based on molecular beacon (MB) probe was developed to detect ochratoxin A (OTA) in malts, which is a common starting material in the brewery industry. With the critical site for OTA binding in capture aptamer in mind, a MB probe containing 20 bases with a fluorophore-quencher pair at the stem ends was designed and synthesized. In the "off"configuration, the fluorescein (FAM) is internally quenched due to close contact with dabcyl and the fluorescence signal is recovered after hybridization with the capture aptamer at the "on" state. In the presence of OTA, the MB probe competes for binding at the loop region of the aptamer, resulting in a decrease in fluorescence signal. Using this indirect competitive assay, the detection of OTA in malt samples was accomplished for the first time. In addition, the effect of binding affinity of the capture aptamer and OTA on the assay performance was investigated. Under optimal conditions, this method allowed for OTA detection at a linear range of 0.0001-1 $\mu g \cdot mL^{-1}$ (correlation coefficient, R=0.9920) with superior sensitivity and a detection limit as low as 0.05 $ng \cdot mL^{-1}$. The sensing system also displayed an excellent selectivity and perfect anti-interference capacity in the matrix. Moreover, the entire process of detection was accomplished in less than 20 min. The recovery from spiked malt samples ranged from 81.0% to 95.2% with RSDs below 5.4%. The performance of our method was further validated by ultra-fast liquid chromatography coupled with tandem mass spectrometry. Compared with similar fluorescence assays, the proposed method is simple, efficient and does not require complicated conjugation steps. Taken together, this novel detection strategy could be a promising tool for hand-held devices used during on-site monitoring of contaminants.

Keywords: molecular beacon (MB); probe; ochratoxin A (OTA); indirect competitive assay; malt; fluorescence assay;

1 Introduction

Ochratoxin A (OTA), primarily produced by the species of *Aspergillus* and *Penicillium*, is one of the important mycotoxins that has been identified as a contaminant in a variety of food products such as wheat, corn, wine, coffee, and so on[1, 2]. OTA can also travel through the food chain by way of animal feed[3, 4]. Many studies have indicated that OTA is a strong nephrotoxic agent[5] that possesses immunotoxicity[6] and teratogenicity[7], and carcinogenic effects[8]. Due to this prevalence and potential toxicity, European authorities have set the maximum residue levels (MRLs) of OTA at 5 $\mu g \cdot kg^{-1}$ for cereals and at 2 $\mu g \cdot mL^{-1}$ for the beverage of wine and beer[9].

Along with setting limited standards, the detection of OTA also plays an essential role in monitoring the levels of OTA in food products. Some of the widely accepted methods for the screening of OTA include high liquid chromatography coupled to fluorescence detection (HPLC- FLD)[10-13] and ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS)[14-16]. Although the above methods can produce excellent results both quantitatively and qualitatively, their applications rely on tedious and costly pre-treatment with immunoaffinity column, expensive equipment, and trained personnel. More recently, sensors based on biomolecules (antibodies and aptamers) or artificial molecules (molecularly imprinted polymer) have become the preferable choice of OTA detection due to their miniature sizes and portability for on-site detection[17-20].

Using aptamers as the recognition elements, aptasensors offer significant advantages in extraordinary selectivity and sensitivity, together with low preparation cost[21-26]. In fact, the pursuit of versatile aptasensors has been continuing an ongoing effort in the field of food safety. To enhance their sensitivity in complex samples, most reported detection schemes focused on the amplification of signals emitted by nanomaterials such as gold nanorods[27] or gold nanoparticles[28], quantum dots[29], silver nanoclusters[30], magnetic beads[31], etc. However, these approaches are often accompanied by obstacles such as complicated process of conjugation, poor photo-stability in harsh matrices and high cost. Leakage of heavy metals from nanomaterials may also produce potential nanotoxicity. Besides developing excellent nanoscaled aptasensors, it is also important to explore other

[a] Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100193, China
[b] Hainan Branch Institute of Medicinal Plant Development, Chinese Academy of Medicinal Sciences & Peking Union Medical College, Wanning 571533, Hainan, China
*Corresponding author: E-mail: yangmeihua15@hotmail.com; Fax: +86 10 62896288; Tel: +86 10 57833277

simple, cost-effective and sensitive sensing approaches for on-site monitoring of contaminants. Molecular beacon (MB), first reported by Tyagi[32], received much attention in the field of life science for its potential in diagnosis of cancer cells[33], proteins[34] and genes[35]. Owing to advantages like facile synthesis and high selectivity, the MB probe has attracted a great deal of interest in contaminants screening for food safety and environmental testings[36, 37]. MB is composed of a loop section and two complementary stems tagged with fluorophore and quencher, respectively[38]. The hairpin structure allows for an on state signal in the presence of a specific oligonucleotides sequence complementary to the loop sequence of the MB probe; while also generates an off state when a target competitively binds to the complementary oligonucleotides sequence. Based on this working principle, the level of targets can be translated into a measurable response. Until recently, with the exception of an analysis by a real-time PCR thermal cycler with a monitoring time of 1 h[39], few studies on OTA screening using the aforementioned strategy have been reported .

In this paper, we designed a double-labelled MB probe, which contains a complementary sequence against a capture aptamer (anti-OTA aptamer). This novel strategy for OTA screening involves a competitive aptamer-binding method that introduces the designed MB probe as a tracer. If a sample contains OTA, the molecules within will compete with the tracer for the aptamer binding sites and the fluorescence signal will decrease. Hence, the probe competitively binds the anti-OTA aptamer, which then inhibits its binding to OTA. This ultimately allows for the detection of OTA even at trace level. This newly developed MB has great potential in on-site monitoring of contaminants in real life samples due to its superior sensitivity, selectivity, simple procedure and low cost.

2 Experimental

2.1 Materials

Mycotoxins standards including aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2), ochratoxin A (OTA), citrinin (CIT), deoxynival enol (DON), zearalenone (ZAN), zearalenone (ZON), fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) were purchased from Pribolab (Singapore). The DNA oligonucleotides were custom-synthesized, labelled, and purified by Sangon Biotech (Shanghai, China). The DNA aptamer against OTA had the following sequence: 5'-GATCGGGTGTGGGTG GCGTAAAGGGAGCATCGGACA-3'. The molecular beacon had the sequence: 5'-FAM-CCGGGTCCACCCACACCCGG-DABCYL-3'. Methanol was HPLC grade. All other reagents were of analytical grade. Without further statement, the buffer solution containing 10 mM Tris-HCl (pH 8.5), 120 mM NaCl, 20 mM $CaCl_2$ and 5 mM $MgCl_2$ was freshly prepared. Three batches of malts with no visible mold contamination, along with two moldy samples due to inappropriate storage, were collected from herbal markets in China.

2.2 Instruments

Fluorescence measurements were performed using an F-7000 fluorescence spectrophotometer (Hitachi, Japan) equipped with FL solutions 2.1 software. The fluorescence spectra were collected with an emission wavelength within the range of 500 nm and 630 nm by setting an excitation wavelength at 495 nm. Slits for the excitation and the emission measurements were both at 5 nm in width. Fluorescence intensities were recorded at 520 nm. The ultrafast liquid chromatography (UFLC) analysis was conducted on a CAPCELL CORE C_{18} column (50 × 2.1 mm, 2.7 μm; Shiseio, Japan) using a binary mobile phase composed of 0.1% (*v/v*) formic acid in acetonitrile (A) and 0.1% (*v/v*) formic acid in water (B) with the following gradient elution program: 0-4 min, linear gradient from 70% B to 40% B; 4-5 min, from 40% to 5%; from 5-7 min, the composition of B was kept at 5%; 7-8 min, the composition of B was returned to 70% and then left for 2 min to equilibrate the column. The flow rate and injection volume was 0.3 $mL \cdot min^{-1}$ and 2 μL, respectively. An Applied Biosystems Sciex QTRAP®5500 MS/MS spectrometer was used to confirm that the positive samples were contaminated with OTA by detecting ion transition of 404.2/239.1 and 404.2/358.0, the former ion transition was also used for quantification.

2.3 Fluorescence measurements

Measurements of OTA based on fluorescence intensity of MB was performed by the following procedure. Incubation of 1 μM of aptamer with various concentrations of OTA in buffer solution for 15 min, followed by addition of 1 μM of MB to the above solution and incubation for 2 min prior to assessment of fluorescence intensity. All measurements were performed at room temperature.

2.4 Sample preparation

A 2 g sample of malt powder was accurately weighed in a 10 mL teflon centrifuge tube. After addition of 5 mL of methanol/water (80/20, *v/v*), the mixture was vigorously vortexed for 1 min and then subjected to ultrasonic extraction for 5 min. The extract was then centrifuged for 5 min at 4000 rpm and the supernatant was diluted 10 times using buffer solution. Finally, the prepared sample was filtrated through a 0.22 μm membrane.

3 Results and discussion

3.1 Principle of operation of molecular beacon probe

In this work, a MB probe is used as a tracer in the fluorescence assay. The MB is a single-stranded oligonucleotide hybridization probe that consists of a stem-and-loop structure. In this particular configuration, the loop contains a probe sequence that is complementary to the capture aptamer, while the stem is composed of complementary arm sequences, which is separately labelled with a fluorescein (FAM) and a quencher (DABCYL). By virtue of conformational changes of the MB, the interaction between an anti-OTA aptamer and an OTA target can be translated into a measurable signal. The detailed sensing strategy illustrated in **Fig. 1a** mainly involves three processes. (i) The MB probe exhibits efficient fluorescence quenching as a result of FAM being in close contact with DABCYL. (ii) Upon mixing with an anti-OTA aptamer, the loop section in MB forms a hybrid with the specific sequence within the anti-OTA aptamer, resulting in a structure that is longer and more stable than the stem hybrid. Consequently, the stem

hybrid is forced to dissociate, causing FAM to be distant from DABCYL. As a result, the fluorescence is restored. (iii) In the presence

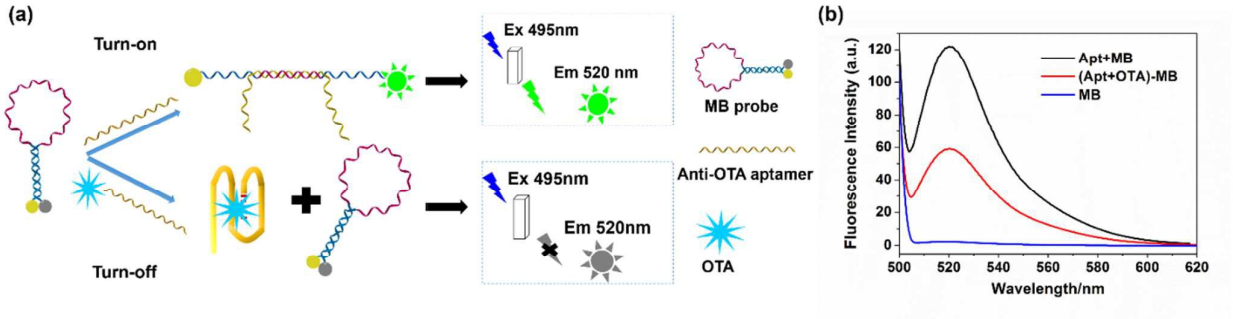


Fig.1 (a) The schematic illustration of the molecular beacon probe for OTA screening; (b) The fluorescence spectra of molecular beacon, aptamer incubated with molecular beacon probe and aptamer reacted with OTA followed by the addition of molecular beacon probe.

of OTA, the anti-OTA aptamer preferentially binds to OTA, leading to a consumption of a certain amount of aptamer. A portion of the MB probes' hairpin structure is therefore, restored to a "closed" configuration and so is their quenching ability. The OTA level in the sample can then be quantified based on the decrease of fluorescence readouts.

For the designed MB probe to function properly under the optimal condition, selection of the appropriate key loop sequence of MB is especially crucial. Zhao et al [40, 41] investigated the different recognition sites of the 36-mer capture aptamer for their binding affinity to OTA and showed that their dissection constant at T10 (from 5' to 3') was approximately that of the whole aptamer. A study by Cruz-Aguado and Penner [42] also demonstrated G11 (from 5' to 3') of the capture aptamer plays a key role in its recognition of OTA. Taking the above studies into consideration, we predicted that the main recognition region of the aptamer to be located at the underlined sequence (5'-GATCGGGTGTGGGTGGCGTAAAGGG AGCATCGGACA-3'), which was shown to possess affinity and specificity for OTA. We therefore, incorporated a corresponding sequence in our MB (complementary to the underlined sequence on the aptamer), which would compete with OTA for binding to the aptamer. According to previous studies, a typical MB contains a loop sequence of 15-30 nucleotides and a stem of 4-8 base pairs. With these previous findings in mind, a MB composed of an 8-mer loop and 6-mer bases in each stem, along with FAM and Dabcyl modifications, was designed and synthesized. To test for changes in fluorescence signal of the designed MB in response to binding of capture aptamer to its target, fluorescence spectra were recorded for 1 $\mu g \cdot mL^{-1}$ of OTA incubated with or without aptamer (the control group). **In Fig. 1b**, the test group exhibited a dramatic decrease of fluorescence intensity after the addition of OTA compared to the control group. The negligible background intensity of the MB and the 100-fold enhancement in fluorescence intensity in control group further indicated that the designed MB functioned properly. Our approach showed that it is feasible to monitor OTA level using the MB probe.

3.2 Optimization of detection conditions

Experimental parameters such as test solution, competitive approach and reaction time, could have a significant impact on binding affinity of the aptamer for OTA, thereby, affecting the accuracy of fluorescence detection.

Based on previous studies[42, 43], alkaline pH and divalent cations (eg. Ca^{2+}) could contribute to the interaction between aptamer and OTA. It has been demonstrated that 10 mM Tris-HCl (pH 8.5), 120 mM NaCl, 5 mM KCl and 20 mM $CaCl_2$ could be used as optimized binding buffer in the application of similar schematic aptamer-based detection for OTA[18, 42-47]. The composition of the buffer solution used in this experiment is based on these previous findings.

It is crucial to investigate the competitive binding of the capture aptamer by the MB probe versus that by OTA. Two different competitive approaches were tested and the results were shown in **Fig. 2a**. One of them involved allowing the MB probe to react with the aptamer for 2 min prior to adding OTA. In the other approach, the aptamer was incubated with OTA for 15 min, followed by addition of the MB probe. The relative intensity (F/F_0) is used as an indicator for the detection of OTA, in which F and F_0 represent the intensity of the probe in the presence and absence of OTA, respectively. As shown in **Fig. 2a**, relative intensity from the latter scheme appeared to be more sensitive to the same concentration of OTA than that from the former. Our results indicated that adding OTA to the aptamer before mixing in the MB probe could help achieve a lower detection limit. Therefore, we selected the strategy that involved incubating the aptamer with OTA, followed by MB probe as the superior approach to measuring OTA levels.

The time-dependence interaction between OTA and the capture aptamer might also affect the sensitivity of the probe. As shown in **Fig. 2b,** the relative intensity (F/F_0) decreased with increment of reaction time. Incubation with OTA for more than 15 min led to the maximum relative intensity. We concluded that longer incubation time would go against the purpose of rapid screening, therefore, 15 min was chosen as the reaction time for our detection procedure.

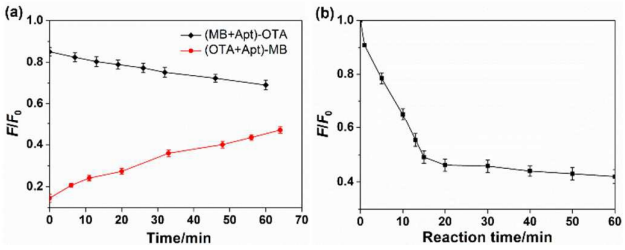


Fig. 2 (a) The relative fluorescence intensity obtained from different competitive styles; (b) the relative intensity changed with reaction time of OTA and the aptamer.

3.3 Selectivity and anti-interference capacity of molecular beacon probe

The robustness of the newly developed probe is a significant criterion for real life application. Various analogues including AF_{sum} (AFB_1, AFB_2, AFG_1 and AFG_2), CIT, DON, ZAN, ZON, FB_1 and FB_2 were separately added to the detection system. As shown in **Fig. 3a,** the relative intensity (F/F_0) of our detection system, followed by addition of the analogues (1 μg·mL^{-1}) alone, generated negligible changes compared with that of the control group, which indicated that the capture aptamer exhibited a high selectivity to OTA. The analogues (1 μg·mL^{-1}) together with OTA (1 μg·mL^{-1}) were incubated with the capture aptamer, followed by the addition of the MB probe. As demonstrated in **Fig. 3b,** none of the analogues led to either an enhancement or an inhibition on the values of F/F_0. In addition, to explore the feasibility of the probe in real samples, the blank malt was selected due to the high occurrence of OTA in those matrices. The anti-interference of the probe was also confirmed (**Fig. 3a and 3b**). Our results indicated that this detection approach responded specifically to OTA and displayed excellent anti-interference capability.

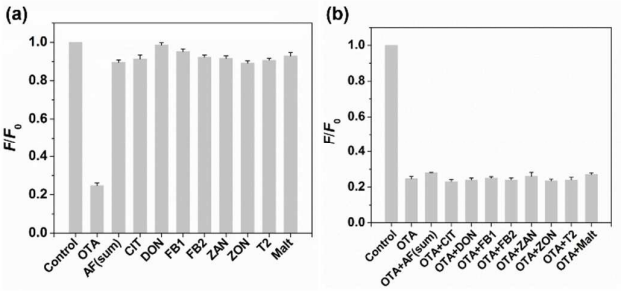


Fig. 3 The relative fluorescence intensity obtained from analogous chemicals and the malt extract (a) without OTA and (b) with OTA. The control group contains only the aptamer and the MB probe. Besides the aptamer and the MB probe, the other groups contain either the analogous (and matrix), or both OTA and the analogous (and matrix).

3.4 Performance of fluorescence assay

3.4.1 Linearity

Opening of the hairpin of the designed MB probe is dependent on the concentration of the capture aptamer. The sensing of OTA was implemented by "consumption" of the aptamer in solution, which resulted in a decrease in fluorescence intensity. To determine whether the fluorescence inhibition of the developed MB probe was proportional to the concentration of OTA in solution, the F values of the probe in the presence of various concentrations of OTA were measured. In the absence of OTA, the fluorescence intensity of the probe was recorded as F_0 in control group. The relative fluorescence intensity (F/F_0) was demonstrated depending on the concentration of OTA over a range of 0.0001-1 μg·mL^{-1} with a good correlation coefficient (R) of 0.9920 (**Fig. 4a and 4b**). These results further confirmed that the established method based on MB probe is a promising approach to quantitatively measure OTA in samples.

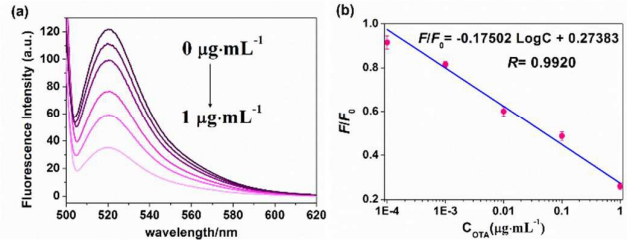


Fig. 4(a) Fluorescence spectra of molecular beacon probe in the presence of serial concentrations of OTA and the capture aptamer; (b) the linear relationship between the relative intensity and the level of OTA.

3.4.2 Sensitivity

Due to the trace amount of contaminants in matrix, most approaches for contaminants detection are required to possess high sensitivity. To evaluate the sensitivity of the MB probe, the limit of detection (LOD) was determined by gradually diluting the standard solution with detection buffer. As a result, using a value of F/F_0 approximated to 5% as the criterion, the LOD was measured to be as low as 0.05 ng·mL^{-1} (corresponding to 1.25 μg·kg^{-1} in malt sample), which is two orders of magnitude lower than the maximum limit for OTA set by the European regulation in wine (2 ng·mL^{-1}) and 0.25-fold lower than in cereals (5 μg·kg^{-1}). This indicated that the developed method is a useful tool for screening trace amount of OTA in real life samples.

3.4.3 Recovery

To evaluate the accuracy of the proposed method, three levels of the spiked malts were measured by both the MB assay and the traditional LC-MS/MS method. Three parallel tests were performed for repeatability assessment. The results in **Table 1** showed that recovery of measurements in the range of 81.0% to 95.2% with RSDs below 5.4% were obtained by the proposed probe method. The experimental results from the LC-MS/MS method further demonstrated that the MB assay is capable of detecting OTA in real life samples.

Table 1 The recovery measured by the proposed method versus LC-MS/MS method (*n*=3)

Matrix	OTA added (μg)	MB probe		LC-MS/MS	
		Measured value ± SD (μg)	Mean Recovery % (RSD%)	Measured value ± SD (μg)	Mean Recovery % (RSD%)
Malt	0.50	0.453±0.016	90.6 (3.5)	0.565±0.015	113.0(2.6)
	0.05	0.048±0.003	95.2 (5.4)	0.054±0.001	107.0(1.5)
	0.01	0.008±0.0003	81.0 (3.8)	0.012±0.004	117.7(3.6)

3.5 Comparison with other similar fluorescence assays

The proposed MB method has two advantages compared with similar fluorescence assays (**Table 2**). First, it allows for the detection of OTA as low as 0.05 ng·mL^{-1}, indicating that the sensitivity of this approach is higher than those of methods based on single-label aptamer[41], dyes PicoGreen[43], HRP-mimicking DNAzyme hairpin[48], and gold nanoparticles[49]. Although some groups reported that OTA can be detected at the picomole level, those assays involved time-consuming conjugation procedures, which goes against the purpose of rapid detection[30]. Although fluorescein-labelled OTA aptamer can rapidly screen for OTA within 1 min, this method is dependent on more than one DNA probe, and therefore, its performance can be affected by multiple factors[44]. Since the MB probe adopts an "open" configuration in the absence of the target while exhibiting a "closed" configuration in the presence of OTA, it allows for the elimination of tedious optimization processes aimed to preserve the appropriate affinity and specificity of the anti-OTA aptamer[50, 51]. Therefore, relative to other existing assays, our method provides a simple and straightforward approach to OTA detection in complex samples.

Table 2 Comparison with other similar fluorescence assays

Transduction system	Remarks	LOD (ng/mL)	Assay time	Matrices	Ref.
Single fluorophore-labelled aptamer	Conjugation-free	0.4	unspecific	wine	33
Aptasensor using dyes PicoGreen	Conjugation-free	1	30min	beer	43
HRP-mimicking DNAzyme hairpin	Conjugation-free	1	70min	wine	37
Salt-induced aggregation of unmodified AuNPs	Conjugation-free	8.1	5min	detection solution	49
Fluorescent DNA-scaffolded silver-nanocluster	At least 2h conjugation procedure	2.1×10^{-3}	15min	wheat	30
Fluorescein-labelled OTA aptamer and quencher-labelled complementary sequence	Conjugation-free; need two DNA probes	0.8	1min	corn	44
Fluorescence platform based on molecular beacon	Conjugation-free	0.05	20min	malt	This work

3.6 Measurement of OTA in real samples

Previous studies reported that high occurrence of OTA in malts presents a risk for human exposure to OTA through the widespread consumption of beer[52, 53]. Malts are often used as starting materials in brewery industry where mold is often found[54]. Consequently, malt was selected for this study. In order to verify the feasibility of the probe detection method in practical applications, malt samples were investigated three times using the MB probe. Two batches of the samples were detected to be contaminated with OTA. The contaminant levels were calculated by the linear regression equation. Our results revealed that the OTA levels in these two positive malt samples were at 4.48±0.24 μg·kg^{-1} and 6.28±0.31 μg·kg^{-1}, respectively. These measurements were also confirmed by the traditional LC-MS/MS method (Fig. S1 in Supplementary Information). Detection of OTA in the malt samples demonstrated that the proposed method is potentially applicable for the monitoring of OTA in complex matrix. Furthermore, it suggested that this new strategy based on MB probe could be applied to small molecular screening as part of contaminants analysis.

4 Conclusion

In summary, a novel indirect competitive fluorescence assay for OTA detection based on MB probe was designed and developed. The constructed MB consists of 8 nucleotides in the loop section and 6 base pairs in each stem, with modification of FAM and Dabcyl, was synthesized and used as the sensing probe. Due to the specificity of the anti-OTA aptamer, the developed method provides a highly selectivity and sensitivity approach for OTA detection at a limit as low as 0.05 ng·mL^{-1}. This simple method involves a straightforward procedure compared to other analogous optical aptasensors, while still provides excellent detection performance. The successful application of the MB method in detecting contamination in malts indicated that this novel strategy could be utilized as a reliable screening tool for the presence of small molecular in real life samples.

Acknowledgements

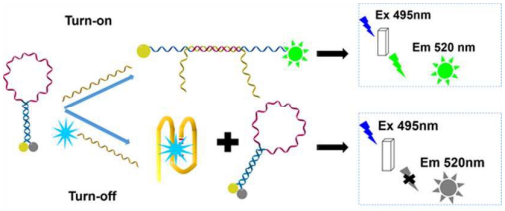
This work was supported by National Natural Science Foundation of China (81274072, 81473346), Specialized Research Fund for Major Science and Technology Program of Hainan, China (ZDZX2013008),

PUMC Youth Fund and the Fundamental Research Funds for the Central Universities (No. 33320140146).

Notes and references

1 T. O. Larsen, A. Svendsen and J. Smedsgaard, *Appl. Environ. Microbiol.*, 2001, **67**, 3630-3635.
2 P. Bayman, J. L. Baker, M. A. Doster, T. J. Michailides and N. E. Mahoney, *Appl. Environ. Microbiol.*, 2002, **68**, 2326-2329.
3 S. T. Sherazi, Z. H. Shar, G. A. Sumbal, E. T. Tan, M. I. Bhanger, H. Kara and S. M. Nizamani, *Mycotoxin Res*, 2015, **31**, 1-7.
4 S. C. Duarte, C. M. Lino and A. Pena, *Vet. Microbiol.*, 2011, **154**, 1-13.
5 A. Mally, G. C. Hard and W. Dekant, *Food Chem. Toxicol.*, 2007, **45**, 2254-2260.
6 L. Al-Anati and E. Petzinger, *J. Vet. Pharmacol. Ther.*, 2006, **29**, 79-90.
7 E. O'Brien, A. Prietz and D. R. Dietrich, *Birth Defects Res, Part B*, 2005, **74**, 417-423.
8 A. Pfohl-Leszkowicz and R. A. Manderville, *Mol. Nutr. Food Res.*, 2007, **51**, 61-99.
9 E. Commission, *Off. J. Eur. Commun. L*, 2005, **25**, 3-5.
10 V. Bascaran, A. H. de Rojas, P. Choucino and T. Delgado, *J. Chromatogr. A*, 2007, **1167**, 95-101.
11 X. Lai, C. Ruan, R. Liu and C. Liu, *Food Chem.*, 2014, **161**, 317-322.
12 M. Solfrizzo, G. Panzarini and A. Visconti, *J. Agric. Food Chem.*, 2008, **56**, 11081-11086.
13 J. Wen, W. Kong, Y. Hu, J. Wang and M. Yang, *Food Control*, 2014, **43**, 82-87.
14 A. Roland, P. Bros, A. Bouisseau, F. Cavelier and R. Schneider, *Anal. Chim. Acta*, 2014, **818**, 39-45.
15 M. Reinsch, A. Töpfer, A. Lehmann, I. Nehls and U. Panne, *Food Chem.*, 2007, **100**, 312-317.
16 Q. Liu, W. Kong, W. Guo and M. Yang, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, 2015, **988**, 175-181.
17 X. P. Liu, Y. J. Deng, X. Y. Jin, L. G. Chen, J. H. Jiang, G. L. Shen and R. Q. Yu, *Anal. Biochem.*, 2009, **389**, 63-68.
18 L. Sheng, J. Ren, Y. Miao, J. Wang and E. Wang, *Biosens. Bioelectron.*, 2011, **26**, 3494-3499.
19 J. Lee, C. H. Jeon, S. J. Ahn and T. H. Ha, *Analyst*, 2014, **139**, 1622-1627.
20 J. G. Pacheco, M. Castro, S. Machado, M. F. Barroso, H. P. A. Nouws and C. Delerue-Matos, *Sensors Actuators B: Chem.*, 2015, **215**, 107-112.
21 A. B. Iliuk, L. Hu and W. A. Tao, *Anal. Chem.*, 2011, **83**, 4440-4452.
22 H. Z. He, D. S. H. Chan, C. H. Leung and D. L. Ma, *Nucleic Acids Res.*, 2013, **41**, 4345-4359.
23 D. L. Ma, H. Z. He, K. H. Leung, H. J. Zhong, D. S. H. Chan and C. H. Leung, *Chem. Soc. Rev.*, 2013, **42**, 3427-3440.
24 H. Z. He, K. H. Leung, H. Yang, D. Shiu Hin Chan, C. H. Leung, J. Zhou, A. Bourdoncle, J. L. Mergny and D. L. Ma, *Biosens. Bioelectron.*, 2013, **41**, 871-874.
25 H. Z. He, V. Pui Yan Ma, K. H. Leung, D. Shiu Hin Chan, H. Yang, Z. Cheng, C. H. Leung and D. L. Ma, *Analyst*, 2012, **137**, 1538-1540.
26 D. Li, S. Song and C. Fan, *Acc. Chem. Res.*, 2010, **43**, 631-641.
27 M. Heurich, M. K. A. Kadir and I. E. Tothill, *Sensors Actuators B: Chem.*, 2011, **156**, 162-168.
28 R. Xiao, D. Wang, Z. Lin, B. Qiu, M. Liu, L. Guo and G. Chen, *Anal. Methods*, 2015, **7**, 842-845.
29 J. Qian, K. Wang, C. Wang, M. Hua, Z. Yang, Q. Liu, H. Mao and K. Wang, *Analyst*, 2015, **140**, 7434-7442.
30 J. Chen, X. Zhang, S. Cai, D. Wu, M. Chen, S. Wang and J. Zhang, *Biosens. Bioelectron.*, 2014, **57**, 226-231.
31 J. Zhang, X. Zhang, G. Yang, J. Chen and S. Wang, *Biosens. Bioelectron.*, 2013, **41**, 704-709.
32 S. Tyagi and F. R. Kramer, *Nat. Biotechnol.*, 1996, **14**, 303-308.
33 X. H. Peng, Z. H. Cao, J. T. Xia, G. W. Carlson, M. M. Lewis, W. C. Wood and L. Yang, *Cancer Res.*, 2005, **65**, 1909-1917.
34 T. Heyduk and E. Heyduk, *Nat Biotech*, 2002, **20**, 171-176.
35 G. T. Hwang, Y. J. Seo and B. H. Kim, *J. Am. Chem. Soc.*, 2004, **126**, 6528-6529.
36 T. M. Lerga and C. K. O'Sullivan, *Anal. Chim. Acta*, 2008, **610**, 105-111.
37 C. Zhang, L. Wang, Z. Tu, X. Sun, Q. He, Z. Lei, C. Xu, Y. Liu, X. Zhang, J. Yang, X. Liu and Y. Xu, *Biosens. Bioelectron.*, 2014, **55**, 216-219.
38 W. Chen, G. Martinez and A. Mulchandani, *Anal. Biochem.*, 2000, **280**, 166-172.
39 S. M. Sanzani, M. Reverberi, C. Fanelli and A. Ippolito, *Toxins (Basel)*, 2015, **7**, 812-820.
40 Q. Zhao, Q. Lv and H. Wang, *Anal. Chem.*, 2014, **86**, 1238-1245.
41 Q. Zhao, X. Geng and H. Wang, *Anal. Bioanal. Chem.*, 2013, **405**, 6281-6286.
42 J. A. Cruz-Aguado and G. Penner, *J. Agric. Food Chem.*, 2008, **56**, 10456-10461.
43 Z. Lv, A. Chen, J. Liu, Z. Guan, Y. Zhou, S. Xu, S. Yang and C. Li, *PLoS One*, 2014, **9**, e85968.
44 J. Chen, Z. Fang, J. Liu and L. Zeng, *Food Control*, 2012, **25**, 555-560.
45 J. A. Cruz-Aguado and G. Penner, *Anal. Chem.*, 2008, **80**, 8853-8855.
46 Z. Guo, J. Ren, J. Wang and E. Wang, *Talanta*, 2011, **85**, 2517-2521.
47 L. Lv, C. Cui, C. Liang, W. Quan, S. Wang and Z. Guo, *Food Control*, 2016, **60**, 296-301.
48 C. Yang, V. Lates, B. Prieto-Simon, J. L. Marty and X. Yang, *Biosens. Bioelectron.*, 2012, **32**, 208-212.
49 C. Yang, Y. Wang, J. L. Marty and X. Yang, *Biosens. Bioelectron.*, 2011, **26**, 2724-2727.
50 N. Li and C. M. Ho, *J. Am. Chem. Soc.*, 2008, **130**, 2380-2381.
51 N. Li, *Journal of the Association for Laboratory Automation*, 2010, **15**, 189-197.
52 A. Visconti, M. Pascale and G. Centonze, *J. Chromatogr.*

A, 2000, **888**, 321-326.
53 R. Mateo, A. Medina, E. M. Mateo, F. Mateo and M. Jimenez, *Int. J. Food Microbiol.*, 2007, **119**, 79-83.
54 S. Běláková, K. Benešová, R. Mikulíková and Z. Svoboda, *Food Chem.*, 2011, **126**, 321-325.



A novel, simple and efficient method based on molecular beacon probe was developed to detect ochratoxin A.