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Dual Aptamer-DNAzyme based colorimetric assay for the detection of AFB1 from food and environmental samples

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ABSTRACT

In the present study, a colorimetric biosensor strategy is devised in combination with apta-magnetic separation assisted with DNAzyme based colorimetric detection of Aflatoxin B1 (AFB1). The optimized analytical procedures consisted of the capture of AFB1 by biotinylated aptamer conjugated to streptavidin magnetic beads and detection by a colorimetric signal from a DNAzyme modified aptamer in presence hemin and H2O2/TMB (3′, 3′, 5, 5′– tetramethylbenzidine). The DNA concentration, incubation time, hemin, and NaCl concentrations were evaluated and optimized. The visual optical signal thus generated could determine the presence of AFB1 in the given sample. The selectivity of the method with other mycotoxins was evaluated. The linear range of AFB1 from 0 to 200 ppb was assessed and detected as low as 40 ppb visually. The absorbance of blue color generated by the catalytic reaction was in a linear correlation with AFB1 concentrations and was able to detect as low as 22.6 ppb (LOD). The suitability of the assay for AFB1 quantification in sorghum and natural samples was also evaluated. Thus, the developed assay could be a reliable, inexpensive, alternative tool for possible use as a screening method for aflatoxins and other mycotoxins.

1. Introduction

Food contamination by mycotoxins is significantly growing public concern is food safety and security due to morbidity, mortality and economic burden. Among food mycotoxins, aflatoxins (AF) are the principal mycotoxins with perilous impacts on human as well as animal health and listed as most potent naturally occurring carcinogens [1,2]. The four major known aflatoxins are Aflatoxin B1, B2, G1 and G2 produced by *Aspergillus* species. Among them, AFB1 is found to be most prevalent aflatoxin in corn, groundnuts, dried fruits, cereals, spices and wide varieties of agriculture and food commodities [3]. It has been estimated that AFB1 through food and polluted air has negatively affected up to 5 billion people in warm and humid climates with frequent outbreaks [4,5]. Aflatoxin B1 contamination is strongly associated with incidences of liver/lung cancer, malaria, growth stunting and childhood malnutrition (kwashiorkor) and bigger risk of adverse birth defects in Asia, Africa, and Central America [1,6]. Additionally, major revenue losses in food and agricultural industry are attributed to crop damage by aflatoxin presence [7,8].

As a preventive measure, several countries have imposed stringent regulations in monitoring and surveillance of AFB1 levels in food. Food Safety and Standards Authority of India (FSSAI), Food and Drug Administration (FDA) and European Union (EU) has set up the maximum of 15–30, 20 and 2 ppb (parts per billion) of AFB1 respectively, as permissible levels in crop and food products [8].

The official analytical methods are based on chromatography principles such as TLC, HPLC and UHPLC–MS/MS, which utilizes cutting-edge technology for accurate multi-mycotoxin detection in a wide range of food matrices [9]. But, chromatographic methods require sophisticated equipments and trained personnel. In such instances immunoassays based on antigen-antibody interactions namely indirect and competitive ELISA, immunoaffinity cleanups (IAC) and immuno sensor are commonly used [10]. These assays show lower limits of detection but they require a stable source of antibodies which are laborious, expensive and time consuming procedures apart from ethical concerns on use of animal models [11]. Moreover all these methods are associated with sophisticated instrument which are limited to well-equipped laboratories [12]. Hence, a simple, cost-effective and fast analytical method as an alternative to traditional technologies and instrumentation methodologies is desirable.

Aptamers are one such attractive alternative ligands developed by an *in vitro* technique known as Systematic evolution of ligands by exponential enrichment (SELEX) [13]. These affinity ligands are single stranded DNA/RNA oligonucleotides that bind to target molecules with

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high affinity and specificity by their unique three-dimensional (3-D) spatial structures and folding patterns [14]. Due to their inherent selectivity and affinity, several assays have been developed for detection of AFB1 [15].

At the same time, catalytic nucleic acids such as DNAzymes have been widely employed as catalytic colorimetric component in biosensing platforms [16]. DNAzymes are G-rich single strand DNA (ssDNA) sequences that can fold into a four-stranded structures known as G-quadruplexes. Some G-quadruplexes have proven to bind hemin and mimic the formation of a catalytic complex with the peroxidase activity. These hemin/G-quadruplexes facilitates a H_2O_2-mediated catalytic oxidation of chromogenic substrate (TMB, ABTS) for colorimetric signal generation [17]. Combination of aptamers and DNAzymes as Functional Nucleic Acids (FNAs) aid in an easy sensing of target by visual color development [18]. Moreover, advantages such as thermal stability and ease of preparation over conventional protein enzymes have attracted great interest in development of biosensor techniques with DNAzymes. Colorimetry is one such commonly used simple optical technique for routine food analysis and this method fulfills the requirements of a simple detection system.

On the other hand, aptamer-conjugated streptavidin magnetic beads based separation technology has emerged as a fast, simple, reliable and effective alternative to conventional immobilization techniques. Occurrence of aflatoxin in complex food matrices hinders their effective detection by any system. Hence, accurate extraction and separation of AFB1 is a must prior to application into any detection platform [19]. Different types of extraction procedures have been reported by many researchers, few involve expensive and complex systems with multiple time consuming steps [20].

On this concern, a dual aptamer based colorimetric assay for capture and subsequent detection of AFB1 was designed. The assay consists of aptamer-based one-step separation of AFB1 by biotinylated (Btn-AFLA5) conjugated to streptavidin-coated magnetic beads and subsequent detection by HRP-DNAzyme labeled AFLA53 (AFLA53-dnazyme) by generation of colorimetric signal was used. The aptamers obtained from our previous work have been used in the present assay [21]. We have optimized several assay parameters and performance of the assay. The effectiveness of developed assay was evaluated using spiked and naturally contaminated food samples.

2. Materials and methods

2.1. Chemicals and reagents

The aptamers sequences used in the present study are listed in Table 1. All chemicals and mycotoxins were procured from Sigma-Aldrich (St.Louis, USA) unless mentioned otherwise. The aptamer probes were prepared in 20 mM Tris–HCl buffer (pH 7.4) and stored at 4 °C until use. Working standards of aflatoxins and other related mycotoxins (1 mg/mL; 1000 ppb) were prepared in absolute methanol (HPLC grade). The chemicals and solvents used in the study are of chemical grade and are listed in the Supporting Information 1.

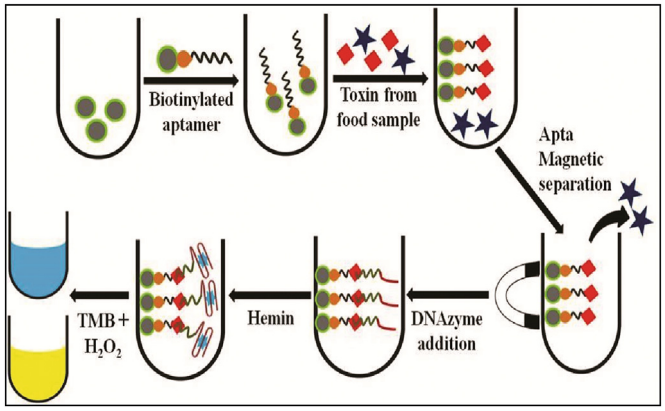

Fig. 1. Schematic illustration of capture and colorimetric detection of aflatoxin B1 by dual aptamer-DNAzyme based assay.

2.2. Strategy for colorimetric biosensor

The colorimetric biosensor was devised using dual aptamers and is illustrated in Fig. 1. The biotin-labeled aptamer (AFLA5) conjugated to streptavidin magnetic beads was used as capture probe for binding of AFB1 [22]. The bound toxin was detected by color development. The colorimetric signal was generated employing DNAzyme modified aptamer (AFLA53) as revealing probe. The two aptamers AFLA5 and AFLA53 consisted of 40 bps and 130 bps, respectively. In the presence of AFB1, formation of the aptamer–analyte complex takes place opening the loop of blocking linker region. As a result, self-assembly of the active HRP-mimicking DNAzyme occurs in presence of hemin and catalytic oxidation reaction of H_2O_2/TMB takes place [16]. The visible blue color formation in the sample mixture confirms the presence of target and can be quantified by the UV–vis spectrometer (TECAN, India) at 450 nm wavelength.

2.3. Optimization of the concentration of capture probe

Aptamer, AFLA5 was used as a capture probe for immobilization of AFB1. The capture probe was biotin-tagged at the 5′ end of the aptamer sequence (Table 1) and synthesized. The optimal concentration of biotinylated AFLA5 (Btn-AFLA5) was investigated for effective capture of the target (AFB1) by ELONA method [21]. Different concentrations of Btn-AFLA5 ranging from 10 to 350 nM were incubated with 200 ppb of AFB1-BSA bound on to microwell plates (Nunc) and enzyme-linked oligonucleotide assay (ELONA) was performed. The resultant absorbance values were measured at 450 nm by TECAN multimode plate reader (TECAN, India).

2.4. Design of revealing probe for colorimetric detection of aflatoxin B1

The hemin/G-quadruplex horseradish peroxidase-DNAzyme (HRP-DNAzyme) has been frequently used as a catalytic nucleic acid label to develop ultrasensitive biosensor and aptasensors. In the present study, AFLA53 labeled HRP-DNAzyme (AFLA53-DNAzyme) was utilized as a revealing probe to detect AFB1 by the visual colorimetric signal. The

Table 1
Oligonucleotide sequences of the aptamers employed in the present study. Aptamer Btn-AFLA5 is biotinylated at the 5′ and AFLA53-DNAzyme consists of DNAzyme sequences represented in italics with small letters showing the poly A linker region.

Name	Oligonucleotide sequence
AFLA5 (Btn-AFLA5)	Biotin- 5′ TTCTTCTGGCTTGGTGGTTGGTGTGTCTGCTGATTTGGTA 3′
AFLA53-DNAzyme	5′CTCGTCTCGTTCTCTCAGTCTGGTTAGCACGGGACCCAGGCTATTGGCAACTTCTAGTCCGACACGAAGAAGAAGGAGGAaaaa *GGTAGGGCGGGTTGGGTAAATaaaaGGTAGGGCGGGTTGGGTAAAT* 3′

sequence of revealing probe comprises of double DNAzyme sequence for colorimetric signal generation, a poly A linker for flexibility, aptamer sequence for binding and capture of aflatoxin B1 (Table 1). Double units of DNAzyme sequences assist in an improved visual colorimetric signal generation.

2.5. Colorimetric assay for determination of aflatoxin (AFB1) employing Dnazymes

The HRP-dnazyme AFLA53 was used in the development of the colorimetric assay. A 50 nM of AFLA53-dnazyme was incubated with, 2 μl of 0.1 mM NaCl and 2 μl of 0.1 μM hemin at 37 °C for 15 min. Followed by incubation, developing solution of freshly prepared H_2O_2/TMB was added and monitored for the development of color. The catalytic oxidation reaction of H_2O_2/TMB in the presence of hemin generated a colorimetric signal that was visible to the naked eye. The absorbance of the color developed was measured with a UV–Vis spectrometer (TECAN, India) at a wavelength of 450 nm.

2.6. Optimization of the concentration of revealing probe

In order to obtain an optimal colorimetric signal, the optimal concentration of revealing probe was investigated. A series of concentrations ranging from 10 to 300 nM were tested with 200 ppb of AFB1 employing DNAzyme mediated colorimetric assay. The intensity of blue color was observed visually and the absorbance values were noted down using UV–Vis spectrophotometer (TECAN, India) at a wavelength of 450 nm.

2.7. Optimization of assay parameters

After optimization of DNA concentrations of capture and revealing probe, assay parameters such as incubation time, hemin, and NaCl concentrations were optimized to achieve precise colorimetric signals. Firstly, the incubation time for the best colorimetric signal was investigated with time intervals ranging from 0 to 40 min. Secondly, hemin and NaCl concentrations ranging from 2 to 10 μM and 10–100 mM, respectively were tested and optimized employing DNAzyme mediated colorimetric assay.

2.8. Apta-magnetic separation (AMS) and capture of aflatoxin B1

The streptavidin-coated magnetic beads (Stp-mag) used in the present study were procured from Thermofisher Scientific, India. A 20 μl of Stp-mag were added to 250 nM Btn-AFLA5 in 100 μl of 1x DPBS and incubated for 30 min at RT with intermittent shaking to induce conjugation. Later on, 200 ppb of AFB1 was added and incubated for 30 min at room temperature. The tubes were then placed on DynaMag Magnet rack (DMR) for 5 min. After aggregation, the supernatant was discarded and 300 μl of 0.2% (w/v) BSA was added and incubated for 30 min at RT to block any excess activated sites. Later on, the tubes were placed on DMR for 5 min, and the supernatant was discarded and aggregates were washed two times with 500 μl of 1x DPBS to remove the unbound toxin.

2.9. DNAzyme mediated colorimetric assay

Dual aptamer system was used in the assay for capture and subsequent detection of AFB1. Initially, apta-magnetic capture of AFB1 was carried out employing Btn-AFLA5. The captured AFB1 was subsequently detected by a visual colorimetric signal with the aid of DNAzyme-AFLA53. The assay is illustrated in Fig. 1. Colorimetric detection of captured AFB1 was carried out by incubating with 100 μl of 50 nM revealing probe, DNAzyme-AFLA53 for 30 min at RT and placed on the DMR for aggregation. Later on, the unbound probe was removed by washing twice with 1x PBST (0.03% of Tween 20). The colorimetric reaction was performed by incubating with 50 μl DPBS buffer, 0.1 mM NaCl, and 0.1 μM hemin at 37 °C for 15 min. For generation of the colorimetric signal, freshly prepared H_2O_2/TMB reagent was added to the solution and color development was monitored. The catalytic reaction of color generation was observed visually and the reaction was terminated with 50 μl H_2SO_4 (0.2 M) and the absorbance at 450 nm was measured with a UV–Vis spectrophotometer (TECAN, India).

2.10. Specificity analysis of apta-magnetic separation assisted colorimetric assay

Specificity and accuracy of the assay were validated in comparison to related and other mycotoxins namely, AFB2, AFG1, AFG2, OTA, FB1, ZEA, CIT, T-2 toxin, DON, and PAT. The mycotoxins of 200 ppb were tested with the DNAzyme mediated colorimetric assay under standardized assay parameters.

2.11. Sensitivity analysis of aptamers-magnetic separation assisted colorimetric assay

The competency of the developed colorimetric assay was investigated with various concentrations of AFB1 (0–200 ppb). To prove the reliability of the developed assay, spiking study was conducted with sorghum. Sorghum samples were procured from local vendors and surface sterilized. The sample was then ground to powder form and tested by HPLC for mycotoxin contamination. Known concentrations of AFB1 from 5 to 100 ppb in 2 ml of DPBS + 5% methanol were added to 5 gm of sorghum samples, respectively. Spiked samples were allowed to air dry and 10 ml of methanol: water (80:20) was added. The mixture was then vortexed and centrifuged at 5000 rpm for 5 min at 4 °C (supporting information 2). The supernatant was collected and subjected to toxin purification by AMS method and subsequent detection by the colorimetric assay.

2.12. Evaluation of apta-magnetic separation (AMS) assisted colorimetric biosensor on food samples and aflatoxin-producing isolates

The practicability and applicability of the developed aptasensor was evaluated by naturally contaminated food samples. Corn (n = 10), rice (n = 4), groundnut (n = 12), black pepper (n = 2) and chilli (n = 3), were obtained from local vendors and tested before performing the assay (Table 2). Toxin extraction from naturally contaminated samples and isolates was carried out. The extraction buffer containing 5 ml of methanol: water (80: 20), 2 ml of hexane was added to contaminated food sample and mixed in a mechanical mixer for 5 min, in case of groundnuts 0.5 gm NaCl was added additionally. The mixture was filtered and the filtrate was evaporated to near dryness under a stream of nitrogen. The extracts were then resuspended in 100 μl of 1x DPBS with 0.2% methanol and tested with the developed biosensor.

2.13. Statistical analysis

All the experiments were carried out in triplicates and data obtained was analyzed. Statistical interpretation of results was carried out with

Table 2
List of food samples analyzed by dual aptamer-DNAzyme based colorimetric assay and found positive for aflatoxin contamination.

Sample name	No. of samples	No. of samples positive for aflatoxin
Corn	10	8
Rice	4	–
Groundnut	12	12
Black pepper	2	1
Chilli	3	2
Total	**31**	**23**

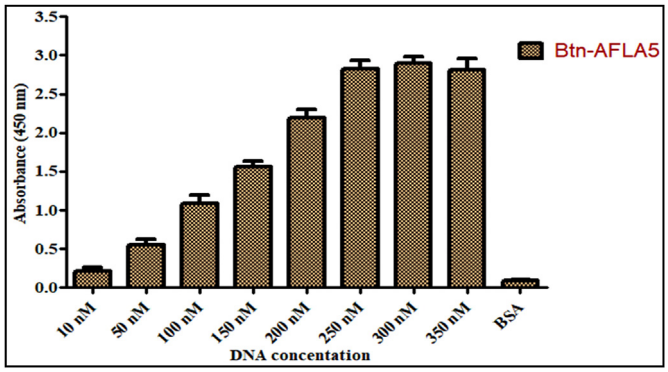

Fig. 2. Optimization of capture probe concentration, (biotin-tagged AFLA5, Btn-AFLA5).

the help of graphpad prism version 5.3 and standard deviation (SD) was obtained and graphs were constructed. The level of significance was set at $p < 0.05$ and the sensitivity was considered at values three times of the blank.

3. Result and discussion

3.1. Optimization of capture probe concentration

Aptamer-conjugated streptavidin magnetic beads based separation technology has emerged as a fast, simple, reliable and effective alternative to conventional immobilization techniques [19]. The biotin-tagged aptamer (Btn-AFLA5) was conjugated to streptavidin-coated magnetic beads and used as a capture probe. The optimal concentration of Btn-AFLA5 for effective capture of the target (AFB1) was confirmed by ELONA. The results suggested, 250 nM of Btn-AFLA5 was effective in binding to 200 ppb of target toxin. As shown in Fig. 2 with the increase of the concentration of capture probe, the absorbance values gradually increased and remained in steady state after 300 nM of DNA. However, the increase in absorbance values at higher DNA concentration (300 nM) was observed which may be a result of imprecise interaction of aptamer with the target or background development [23]. Hence 250 nM of Btn-AFLA5 was selected for the experiment. Furthermore, the assay proves that the central domain of the aptamer plays a major role in target binding and removal of primer regions did not affect the capturing ability.

3.2. Optimization of revealing probe concentration

The aptamer AFLA53 sequence comprising double DNAzyme sequences was used as revealing probe for colorimetric signal generation in presence of AFB1. The optimal concentration of revealing probe (AFLA53-dnazyme) was evaluated for effective colorimetric signal upon detection of AFB1. A series of concentrations (10–100 nM) of AFLA53-dnazyme were tested for generation on a visual blue color. As per the results obtained, 50 nM of revealing probe produced significant blue color by H_2O_2 mediated TMB catalysis, while higher concentrations had little to no effect in color development (Fig. 3). No color development for the double-stranded probe in presence of AFB1 was observed; this proved the binding toxin with single stranded aptamer probe. The presence of a poly A linker assisted in the flexibility of aptamer sequence for target recognition [24]. Additionally, revealing probe comprising of single, double and triple DNAzymes sequences was investigated for optimal blue color generation [25] (data not shown). Double sequence of DNAzyme probe produced an optimal visible in comparison to single and triple sequence probes, which generated either very faint or false positive dark blue color signals, respectively.

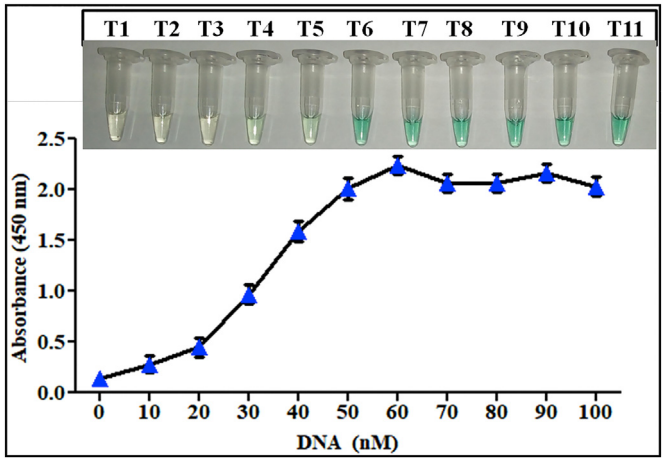

Fig. 3. Optimization of concentration of revealing probe (AFLA53-dnazyme), T1-T11 indicate the different concentration of AFLA53-dnazyme (0–100 nM).

3.3. Optimization of assay parameters for colorimetric detection of AFB1

To attain best colorimetric signal, assay parameters (incubation time, hemin and NaCl concentration) which can influence the sensitivity of the aptasensor were optimized. The colorimetric signal of DNAzymes bound to AFB1 was compared with control under different assay conditions. Firstly, the incubation time for the colorimetric sensing platform was investigated and a strong signal was obtained after 15 min and a further increase in time duration had no effect on signal intensity (Fig. 4a). This strengthened fast detection of toxin by the developed assay.

Later, concentrations hemin and NaCl which also influence the catalysis reaction were investigated. The results suggested (Fig. 4b) that, 50 mM of NaCl generated an effective signal on contrary to higher concentration where a reduction in absorbance signal was observed. At a concentration of 4 μM of hemin, an effective colorimetric signal was generated and it was observed that at higher concentrations, the absorbance signal became unstable and reduced (Fig. 4c).

3.4. Specificity of the aptamagnetic separation (AMS) assisted colorimetric assay

The selectivity of the aptasensor for AFB1 was examined in comparison to related and other mycotoxins. As shown in Fig. 5, the color of the solution turned to blue in the presence of AFB1. The absorbance values of target toxin and aptamer complex were considered as 100 and percentage of reactivity with other mycotoxins was calculated and found to be 37, 15, and 13% with AFB2, AFG1 and AFG2 respectively. Such reactivity was attributed to structural similarities of aflatoxin members; with other mycotoxins the reactivity was less than 6% which was similar to that of negative control [26]. These inferences facilitate the specificity of the aptamer and the developed assay.

3.5. Sensitivity analysis of the aptamagnetic separation (AMS) assisted colorimetric assay

The competency of the developed colorimetric biosensor was evaluated with various concentrations of AFB1 (0–200 ppb) and spiking studies were conducted. As the results suggest, the developed colorimetric assay was able to detect as low as 40 ppb and color developed was visible to the bare eye (Fig. 6). Sorghum samples were spiked with various concentrations AFB1 (5–100 ppb) and toxin extraction was carried out and subjected to standardized AMS purification and colorimetric assay. A linear relationship between absorbance and AFB1 concentrations was observed and R^2 value was found to be 0.9616

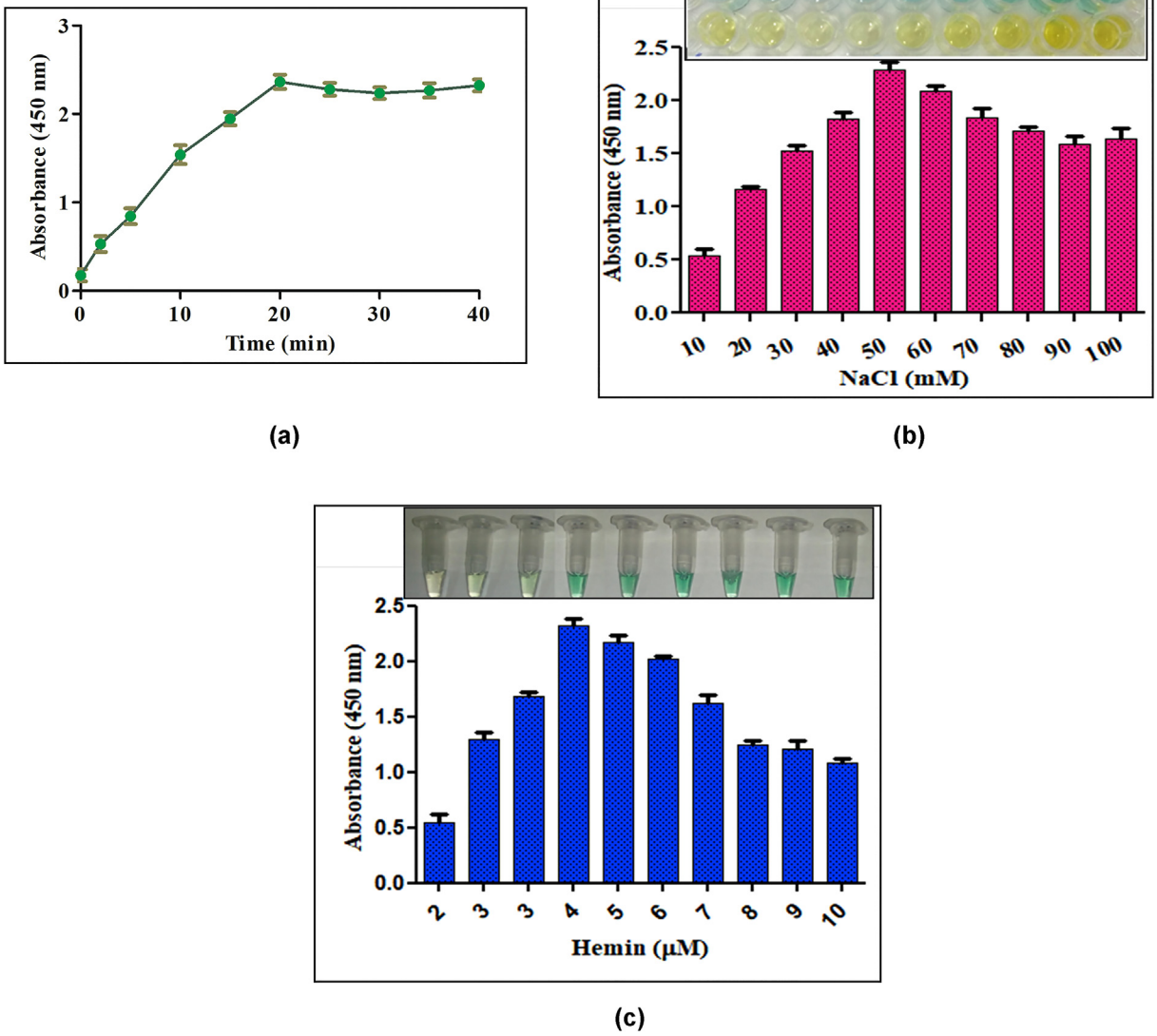

Fig. 4. Optimization of assay parameters for colorimetric detection of AFB1; Fig. 4a. Incubation time; Fig. 4b. NaCl concentration; Fig. 4c. Hemin concentration.

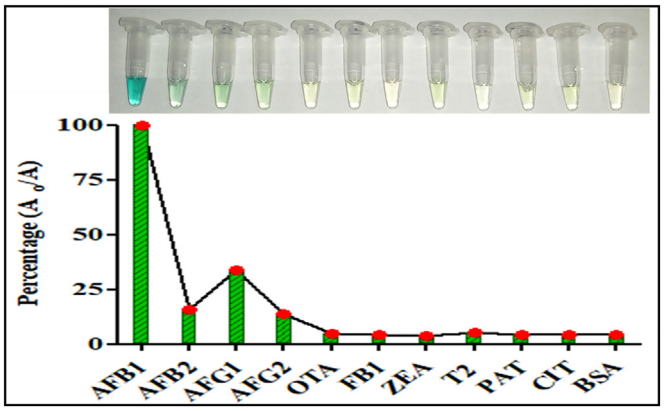

Fig. 5. Specificity analysis of aptamagnetic separation (AMS) assisted colorimetric assay.

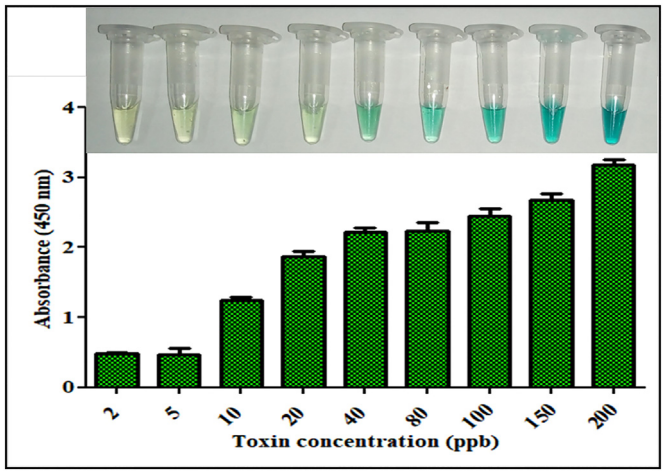

Fig. 6. Sensitivity analysis of aptamagnetic separation (AMS) assisted colorimetric assay.

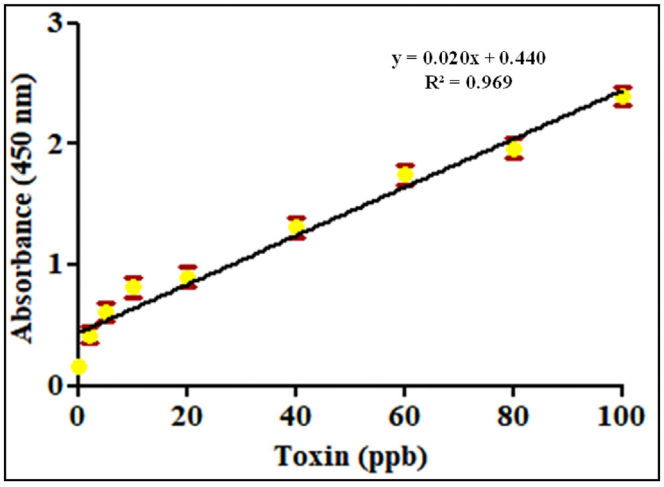

Fig. 7. Calibration curve of absorbance values (nm) versus aflatoxin B1 concentration (ppb) employing aptamagnetic separation (AMS) assisted colorimetric assay.

Table 3
List of fungal isolates analyzed by dual aptamer-DNAzyme based colorimetric assay and found positive for aflatoxin contamination.

S. No	Fungal ID	ITS sequencing	HPLC	DNAzyme assay
1	AFC8	*A. flavus*	+	+
2	AFC13	*A. flavus*	+	+
3	AFC14	*A. flavus*	+	+
4	AFC20	*A. parasiticus*	+	+
5	AFC24	*A. parasiticus*	+	–
6	AFC27	*A. flavus*	+	+
7	AFC 28	*A. flavus*	+	+
8	AFC30	*A. flavus*	+	+
9	AFC31	*A. parasiticus*	+	+
10	AFC33	*A. flavus*	+	+
11	KFC7	*A. parasiticus*	+	+
12	KFC11	*A. parasiticus*	+	+
13	KFC14	*A. flavus*	+	+
14	KFC33	*A. flavus*	+	+
15	KFC32	*A. flavus*	+	+
16	KFC44	*A. flavus*	+	+
17	KFC48	*A. flavus*	+	+
18	KF66	*A. parasiticus*	+	+
19	KFC70	*A. parasiticus*	+	–
20	KFC71	*A. flavus*	+	+
21	TFC1	*A. parasiticus*	+	+
22	TFC4	*A. parasiticus*	+	+
23	TFC6	*A. flavus*	+	+
24	TFC7	*A. flavus*	+	+
25	TFC8	*A. flavus*	+	+
26	TFC13	*A. parasiticus*	+	+
27	TFC15	*A. flavus*	+	+
28	TFC42	*A. parasiticus*	+	+
29	TFC43	*A. parasiticus*	+	+
30	TFC44	*A. parasiticus*	+	+

(Fig. 7). Limit of detection calculated from the linear regression analysis was found to be 22.6 ppb. These observations demonstrated substantial sensitivity of the developed assay towards AFB1 attributing to strong aptamer ligand interaction.

3.6. Purification and detection of AFB1 from food samples

Occurrence of aflatoxin in complex food matrices hinders their effective detection by any system. Hence, accurate extraction and separation of AFB1 is a must prior to application into any detection platform [27]. The robustness of our method was assessed with natural samples and aflatoxin producing isolates. The aptasensor was able to successfully capture and detect AFB1 with visible colorimetric signal from contaminated food samples (23/31 samples) and fungal isolates (28/30 isolates) (Table 2; Table 3). This is attributed to the imprecise and feeble interaction of aptamer with components of food matrix [28]. This insignificant variation could be resolved by keeping a blank from the test sample extracts. These experimental results demonstrated that AMS assisted colorimetric apta-sensor could be as a rapid, easy and low-cost detection platform for AFB1 in food analysis. The colorimetric signal can be detected by the naked eye or by the UV–vis spectrometer. Moreover, the designed strategy can be adopted as a useful tool for other toxins as an alternative capture and detection system as well.

4. Conclusion

In this study, we have reported the development of dual aptamer-DNAzyme based colorimetric assay for the detection of AFB1. The assay relies on the formation of a sandwich-like assemble between the target and two target-specific aptamers. The AFB1 captured by apta magnetic beads was detected by DNAzyme linked aptamers and a colorimetric signal was detected and quantified. The obtained results establish that the developed biosensing platform has high stability and specificity. This aptamer-based purification and optical detection system might be extended to detect multiple mycotoxins and also other targets molecules directly in the field.

Author's contribution

Keerthana Setlem: Conceptualization, Methodology, Data curation, Writing - Original draft preparation, Investigation, Visualization, Writing- Reviewing and Editing.

Bhairab Mondal: Methodology.

Dr. Shylaja R: Conceptualization, Visualization, Supervision, Writing- Reviewing and Editing, Validation.

Dr. Manmohan Parida: Supervision.

Declaration of competing interest

The authors declare no financial or commercial conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ab.2020.113874.

References

[1] Godfrey S. Bbosa, David Kitya, A. Lubega, Jasper Ogwal-Okeng, William W. Anokbonggo, David B. Kyegombe, Review of the biological and health effects of aflatoxins on body organs and body systems, Aflatoxins-recent Advances and Future Prospects, InTech, 2013.

[2] IARC, Monograph on the Evaluation of Carcinogenic Risk to Humans vol. 85, World Health Organization, Geneva, 2004, p. 51.

[3] Sharanaiah Umesha, Bhadvelu Chandrasekhar, Prahlad Shivakumara, Jayanna Shiva Kumar, Sri Raghava, Prakasha Avinash, Marahel Shirin, et al., Aflatoxins and food pathogens: impact of biologically active aflatoxins and their control strategies, J. Sci. Food Agric. 97 (6) (2017) 1698–1707.

[4] Ferenc Peles, Péter Sipos, Zoltán Győri, Walter P. Pfliegler, Federica Giacometti, Andrea Serraino, Giampiero Pagliuca, Teresa Gazzotti, István Pócsi, Adverse effects, transformation and channeling of aflatoxins into food raw materials in livestock, Front. Microbiol. 10 (2019).

[5] Jonathan H. Williams, Timothy D. Phillips, Pauline E. Jolly, Jonathan K. Stiles,

Curtis M. Jolly, Deepak Aggarwal, Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions, Am. J. Clin. Nutr. 80 (5) (2004) 1106–1122.
[6] Pradeep Kumar, Dipendra K. Mahato, Madhu Kamle, Tapan K. Mohanta, Sang G. Kang, Aflatoxins: a global concern for food safety, human health and their management, Front. Microbiol. 7 (2017) 2170.
[7] Dipendra Kumar Mahato, Kyung Eun Lee, Madhu Kamle, Sheetal Devi, Krishna Dewangan, Pradeep Kumar, Sang Gu Kang, Aflatoxins in food and feed: an overview on prevalence, detection and control strategies, Front. Microbiol. 10 (2019) 2266.
[8] Ramesh Kumar Sharma, Salvatore Parisi, Pesticides detection and detrimental chemicals in Indian farming. Reasons for EU border rejections, Toxins and Contaminants in Indian Food Products, Springer, Cham, 2017, pp. 55–65.
[9] Campbell Katrina, Ana L. Ferreira Cavalcante, Pamela Galvin-King, Michalina Oplatowska-Stachowiak, Catherine Brabet, Isabelle Metayer, Didier Montet, Simon A. Haughey, Christopher T. Elliott, Evaluation of an alternative spectroscopic approach for aflatoxin analysis: comparative analysis of food and feed samples with UPLC–MS/MS, Sensor. Actuator. B Chem. 239 (2017) 1087–1097.
[10] Ruchika Chauhan, Jay Singh, Tushar Sachdev, T. Basu, B.D. Malhotra, Recent advances in mycotoxins detection, Biosens. Bioelectron. 81 (2016) 532–545.
[11] Boonchoy Soontornworajit, Yong Wang, Nucleic acid aptamers for clinical diagnosis: cell detection and molecular imaging, Anal. Bioanal. Chem. 399 (4) (2011) 1591–1599.
[12] S. Hosseini, P. Vázquez-Villegas, M. Rito-Palomares, S.O. Martinez-Chapa, Advantages, disadvantages and modifications of conventional ELISA, Enzyme-linked Immunosorbent Assay (ELISA), Springer, Singapore, 2018, pp. 67–115.
[13] K.M. Song, S. Lee, C. Ban, Aptamers and their biological applications, Sensors 12 (1) (2012) 612–631.
[14] R.N. Veedu, J. Wengel, Locked nucleic acids: promising nucleic acid analogs for therapeutic applications, Chem. Biodivers. 7 (3) (2010) 536–542.
[15] C.M. Maragos, M. Busman, Rapid and advanced tools for mycotoxin analysis: a review, Food Addit. Contam. 27 (5) (2010) 688–700.
[16] C. Yang, V. Lates, B. Prieto-Simón, J.L. Marty, X. Yang, Aptamer-DNAzyme hairpins for biosensing of Ochratoxin A, Biosens. Bioelectron. 32 (1) (2012) 208–212.
[17] J. Zhu, T. Li, J. Hu, E. Wang, A novel dot-blot DNAzyme-linked aptamer assay for protein detection, Anal. Bioanal. Chem. 397 (7) (2010) 2923–2927.
[18] L. Gong, Z. Zhao, Y.F. Lv, S.Y. Huan, T. Fu, X.B. Zhang, G.L. Shen, R.Q. Yu, DNAzyme-based biosensors and nanodevices, Chem. Commun. 51 (6) (2015) 979–995.
[19] A. Paul, M. Avci-Adali, G. Ziemer, H.P. Wendel, Streptavidin-coated magnetic beads for DNA strand separation implicate a multitude of problems during cell-SELEX, Oligonucleotides 19 (3) (2009) 243–254.
[20] C. Bircan, The determination of aflatoxins in spices by immunoaffinity column extraction using HPLC, Int. J. Food Sci. Technol. 40 (9) (2005) 929–934.
[21] Keerthana Setlem, Bhairab Mondal, Shylaja Ramlal, Joseph Kingston, Immuno affinity SELEX for simple, rapid, and cost-effective aptamer enrichment and identification against aflatoxin B1, Front. Microbiol. 7 (2016) 1909.
[22] S. Wu, N. Duan, Z. Wang, H. Wang, Aptamer-functionalized magnetic nanoparticle-based bioassay for the detection of ochratoxin a using upconversion nanoparticles as labels, Analyst 136 (11) (2011) 2306–2314.
[23] X. Wang, R. Niessner, D. Tang, D. Knopp, Nanoparticle-based immunosensors and immunoassays for aflatoxins, Anal. Chim. Acta 912 (2016) 10–23.
[24] Y. Seok, J.Y. Byun, W.B. Shim, M.G. Kim, A structure-switchable aptasensor for aflatoxin B1 detection based on assembly of an aptamer/split DNAzyme, Anal. Chim. Acta 886 (2015) 182–187.
[25] W.B. Shim, H. Mun, H.A. Joung, J.A. Ofori, D.H. Chung, M.G. Kim, Chemiluminescence competitive aptamer assay for the detection of aflatoxin B1 in corn samples, Food Contr. 36 (1) (2014) 30–35.
[26] C. Cervino, E. Weber, D. Knopp, R. Niessner, Comparison of hybridoma screening methods for the efficient detection of high-affinity hapten-specific monoclonal antibodies, J. Immunol. Methods 329 (1–2) (2008) 184–193.
[27] T.E. Möller, M. Nyberg, Efficiency of different extraction solvent mixtures used in analyses of aflatoxins from a certified peanut meal reference material, Food Addit. Contam. 21 (8) (2004) 781–785.
[28] V. Pichon, F. Brothier, A. Combès, Aptamer-based-sorbents for sample treatment—a review, Anal. Bioanal. Chem. 407 (3) (2015) 681–698.