Aptamer-Based Fluorescence Quenching Approach for Detection of Aflatoxin M₁ in Milk

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Aflatoxin M₁ (AFM₁), one of the most toxic mycotoxins, is a feed and food contaminant of global concern. In this study, we developed a fast and simple method for detection of AFM₁ based on a structure-switching signaling aptamer. This aptasensor is based on the change in fluorescence signal due to formation of an AFM₁/aptamer complex. To generate the aptasensor, the specific aptamer was modified with FAM (carboxyfluorescein), and their complementary DNAs (cDNA) were modified with a carboxytetramethylrhodamine (TAMRA) quenching group. In the absence of AFM₁, the aptamers were hybridized with cDNA, resulting in quenching of the aptamer fluorescence due to the proximity of the aptamer's fluorophore to the quenching group on the cDNA. On the other hand, in the presence of AFM₁, a structural switch in the aptamer was induced by formation of an AFM₁/aptamer complex. Changes in the structure of the aptamer led to the release of the cDNA, causing the generation of a fluorescence signal. Thus, AFM₁ concentrations could be quantitatively monitored based on the changes in fluorescences. Under optimized conditions, this assay exhibited a linear response to AFM₁ in the range of 1–100 ng/mL and a limit of detection of 0.5 ng/mL was calculated. This proposed aptasensor was applied to milk samples spiked with a dilution series of AFM₁, yielding satisfactory recoveries from 93.4 to 101.3%. These results demonstrated that this detection technique could be useful for high-throughput and quantitative determination of mycotoxin levels in milk and dairy products.

Keywords: aflatoxin M1, aptasensor, fluorescence, quenching, food safety

INTRODUCTION

Mycotoxins are highly toxic secondary metabolites and produced by several fungal genera, including *Aspergillus*, *Fusarium*, and *Penicillium* (Li et al., 2012; Liu et al., 2015). To date, more than 400 mycotoxins have been identified (Selvaraj et al., 2015). Among them, aflatoxins (AFs), deoxynivalenol (DON), ochratoxin (OTA), fumonisin B₁ (FB₁), zearalenone (ZEN), and T-2 toxin (a trichothecene mycotoxin) are common mycotoxins (Yang et al., 2017). As the most toxic AF, aflatoxin B₁ (AFB₁) is classified as a group 1 carcinogen by the International Agency for Research on *Cancer* (IARC) (Iqbal et al., 2015). In addition, aflatoxin M₁ (AFM₁) is a hydroxylated

metabolite of AFB_1, and approximately 2–6% of AFB_1 in contaminated animal feed is converted to AFM_1 in milk (Abera et al., 2019). AFM_1 mainly causes chronic hepatitis and liver tumors in humans, which was classified as group 2 and changed to group 1 carcinogen by IARC (Karczmarczyk et al., 2016). Due to the serious harm caused to human and animal health by mycotoxins, many countries and international organizations (e.g., the European Union and the Joint FAO/ WHO Expert Committee on Food Additives) have established relevant standards for limiting AFM_1 contamination in foods to extremely low levels (Wang et al., 2013; Song et al., 2014). In Europe, the maximum residue level of AFM_1 in milk is 0.05 μg/kg (European Commission, 2006). In China and the United States, the maximum tolerated level for AFM_1 in milk is 0.5 μg/kg (Busman et al., 2015; Mao et al., 2015). Once raw milk is contaminated with AFM_1, conventional heat treatment (pasteurization or ultra-high temperature) for milk and dairy products is not easy to degrade AFM_1 (Beitollahi et al., 2020).

Despite strict regulation and control of AFM_1 levels in milk, AFM_1 pollution still occurs frequently (Li et al., 2017; Zheng et al., 2017). Consequently, methods for detecting AFM_1 in milk are attracting increasing public attention. Many methods for detecting AFM_1 have been developed, including high-performance liquid chromatography (HPLC) (Mao et al., 2015; Pietri et al., 2016), enzyme-linked immunosorbent assay (ELISA) (Li et al., 2009; Kav et al., 2011), immunochromatography (Busman et al., 2015), thin-layer chromatography (TLC) (Sassahara et al., 2005), and liquid chromatography coupled with mass spectroscopy (LC-MS)(Vaclavik et al., 2010). However, instrument-based analytical methods suffer from several drawbacks, including the requirements of technical expertize and complicated, time-consuming sample pretreatment, making these approaches unsuitable for field operation(Eivazzadeh et al., 2016). Moreover, antibody-based immunoassay systems require high-quality antibodies, which are often too expensive, unstable, or susceptible to degradation and denaturation for field applications(Istamboulia et al., 2016). In the past few years, several groups of researchers have pursued AFM_1 detection technology on immunoassays combined with fluorescence (Shen et al., 2017), electrochemistry (Parker and Tothill, 2009) or chemiluminescence (Vdovenko et al., 2014). Biosensor-based aptamers have attracted increasing attention due to their numerous advantages, including high sensitivity and specificity, easy synthesis and modification, labeling, reusability, and portability (Rhouati et al., 2016). Malhotra et al. used laminated magnetic beads to select this AFM_1 aptamer by exponential enrichment (SELEX technique). The aptamer has a unique structure that encompasses two overlapping stem loops without conserved motif or G-quadruplex, which has lowest dissociation constant value (K_d = 35 nM). The researchers confirmed that the aptamer has good affinity against AFM_1. In addition, the specific aptamer has previously been applied for AFM_1 sensing for our research team (Malhotra et al., 2014). Guo X et al. developed a biosensor based on this aptamer to detect AFM_1 and combined the advantages of strong recognition ability of the aptamer to AFM_1 and excellent amplification efficiency of the RT-qPCR technique to improve

sensitivity (Guo et al., 2016). Recently, Kordasht H et al. used cyclic voltammetry and differential pulse voltammetry techniques to a bioassay based on this aptamer for the determination of AFM_1 (Kordasht et al., 2019).

In particular, structure-switching fluorescence-quenching signal adaptation platforms are considered promising approaches for detection of various biomolecules due to their high sensitivity and specificity, and the fluorescence-quenching approach has been used for the detection of AFB_1 in our previous research(Chen et al., 2017). Sharma et al. developed a structure switching aptamer assay for AFM_1 detection. However, the selectivity of the aptamer for AFM_1 was not clear since only irrelevant OTA and AFB_1 were chosen as interferences to study the cross-reaction (Sharma et al., 2016). Cross-activity tests between other toxins (especially the structural analogs AFB_1, AFB_2, AFG_1, and AFG_2) should be carried out to answer the question whether the aptasensor is suitable for quantifying the AFM_1 concentration in real samples. In addition, the coexistence of multiple mycotoxins in foods is a common and complex phenomenon. Therefore, the effect of mix-mycotoxins on the specificity of the aptasensor should be investigated.
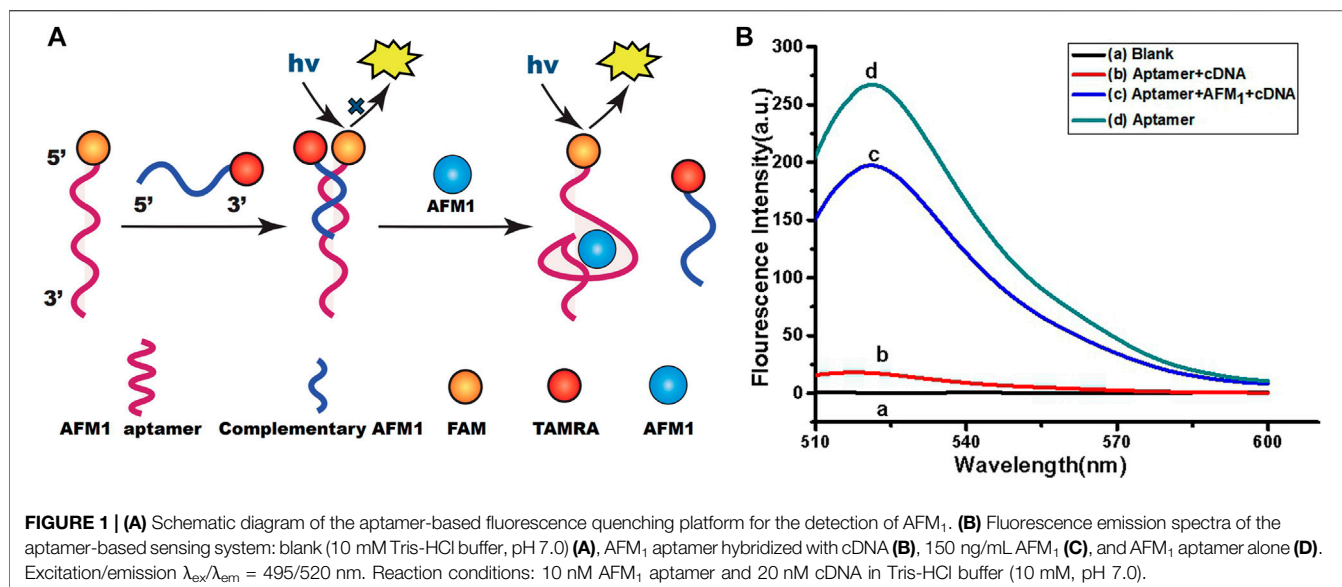
Thus, we developed the biosensor for the detection of AFM_1 based on the specific aptamer, as well as the study of optimal aptamer complementary DNA and structure conversion signal. In the specificity experiment part, we detected the cross-reaction of AFB_1, AFB_2, AFG_1, AFG_2, OTA, ZEN, FB_1, and mix-mycotoxins for the aptasensor performance. In addition, this proposed aptasensor was confirmed to be simple, fast, convenient, economical and effective. The method represents a new practical application of aptamers for the detection of AFM_1 in milk and other dairy products.

MATERIALS AND METHODS

Materials and Reagents

AFM_1, AFB_1, aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), OTA, ZEN, and FB_1 were purchased from Qingdao Pribolab Pte. Ltd. (Qingdao, China). The Tris-HCl buffer (pH 7.0) used in this study contained 10 mM 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris), 120 mM sodium chloride (NaCl), 5 mM potassium chloride (KCl), and 20 mM anhydrous calcium chloride ($CaCl_2$). All chemical reagents were obtained from Shanghai Chemical Reagent Company (Shanghai, China). Phosphate-buffered saline (PBS) was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Liquid whole milk samples were purchased from a local supermarket (Mengniu Dairy, Beijing, China). Distilled water was purified using a Milli-Q purification system. Measurement of fluorescence was performed in Tris-HCl buffer. AFM_1-aptamer labeled with fluorescein (FAM) and its complementary sequences labeled with carboxytetramethylrhodamine (TAMRA) were synthesized by Wuhan GeneCreate Biological Engineering (Wuhan, China) and purified by HPLC. Their sequences were as follows:

AFM_1 Aptamer (Malhotra et al., 2014):

FIGURE 1 | (A) Schematic diagram of the aptamer-based fluorescence quenching platform for the detection of AFM_1. **(B)** Fluorescence emission spectra of the aptamer-based sensing system: blank (10 mM Tris-HCl buffer, pH 7.0) **(A)**, AFM_1 aptamer hybridized with cDNA **(B)**, 150 ng/mL AFM_1 **(C)**, and AFM_1 aptamer alone **(D)**. Excitation/emission $\lambda_{ex}/\lambda_{em}$ = 495/520 nm. Reaction conditions: 10 nM AFM_1 aptamer and 20 nM cDNA in Tris-HCl buffer (10 mM, pH 7.0).

5′-ATC CGT CAC ACC TGC TCT GAC GCT GGG GTC GAC CCG GAG AAA TGC ATT CCC CTG TGG TGT TGG CTC CCG TAT-FAM-3′

Complementary DNA (cDNA):

cDNA1: 5′-TAMRA- ATA CGG GA-3′
cDNA2: 5′-TAMRA- ATA CGG GAG -3′
cDNA3: 5′-TAMRA- ATA CGG GAG C-3′
cDNA4: 5′-TAMRA- ATA CGG GAG CC-3′
cDNA5: 5′-TAMRA- ATA CGG GAG CCA-3′
cDNA6: 5′-TAMRA- ATA CGG GAG CCA A-3′
cDNA7: 5′-TAMRA- ATA CGG GAG CCA AC-3′

Aptamer-Based Fluorescence-Quenching Assay for AFM_1 Detection

First, the AFM_1 aptamer and cDNA were dissolved and diluted in Tris-HCl buffer, and 500 μL AFM_1 aptamers (10 nM) were mixed with 500 μL cDNA. To optimization, the ratios of AFM_1 aptamer: cDNA were set at 1:1, 1:2, 1:3, 1:4, or 1:5 (unless otherwise stated, experiments used a molar ratio of 1:2). Then, the mixture of AFM_1 aptamer and cDNA was heated at 88°C for 5 min and allowed to stand at room temperature for at least 30 min. Next, 500 μL of different concentrations of AFM_1 standard solution was added to the mixture while vortexing. The final reaction volume was 1.5 mL. Similarly, 500 μL Tris-HCl buffer was added to the mixture of AFM_1 aptamer and cDNA while vortexing without addition of AFM_1 and then the fluorescence intensity of fluorescence quenching was measured. Fluorescence intensities at excitation/emission wavelengths of 495/520 nm were recorded on a F-7000 fluorescence spectrophotometer (Hitachi, Japan). To avoid the influence of aflatoxin background fluorescence signal, we performed background correction by measuring the fluorescence of a control containing AFM_1 and buffer without FAM-modified AFM_1 aptamer or cDNA (Chen et al., 2017).
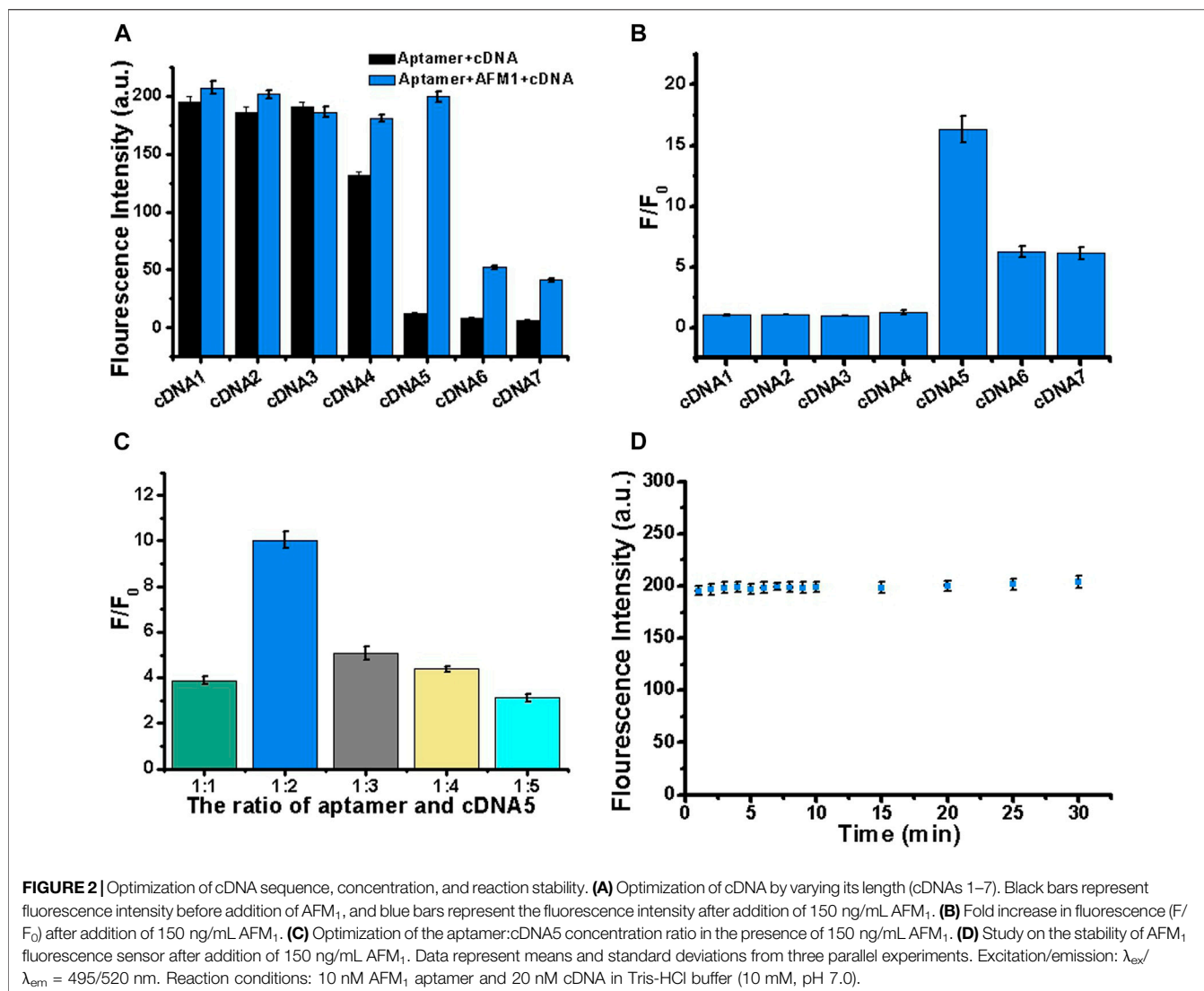
Specificity Analysis

To evaluate the ability of this assay to detect AFM_1 with high specificity, seven mycotoxins structurally related to AFM_1 (AFB_1, AFB_2, AFG_1, AFG_2, OTA, ZEN, and FB_1) were selected for testing. All mycotoxins were used at the same concentration (40 ng/mL). All other detection conditions were identical to those used in the AFM_1 procedure, allowing comparison of fluorescence intensity for all toxins texted.

Real Sample Analysis

The assay was validated for quantitative detection of AFM_1 in liquid milk samples that were spiked with known concentrations of AFM_1. When the concentration of AFM_1 in milk samples was in the range of 1–100 ng/mL, the following pretreatment methods were used. Samples were spiked with AFM_1 at a concentration of 0.5, 5, 10, or 20 ng/mL. Each sample was accurately weighed (0.5 mL) and adding into 10 mL centrifuge tubes, and then 2.5 mL of 70% methanol in water was added to extract AFM_1 from the sample. The entire mixture was vortexed for 5 min on a Vortex-Genie (Scientific Industries, Bohemia, NY, United States), and then centrifuged at $10,000 \times$ rpm for 10 min. The supernatant was collected, filtered through a 0.22-μm filter, and concentrated to 0.5 mL using a N1-Automatic Nitrogen Concentrator (Preekem Scientific, Shanghai, China). Finally, each of the residues was re-dissolved in 2 mL of aqueous methanol solution (5%, v/v) and stored at 4°C until needed. Five replicates were measured for each sample to assess the accuracy of the assay.

When the concentration of AFM_1 in milk samples was in the range of 0–1.0 ng/mL, samples were pretreated to increase sample quantity. The samples accurately weighed (25 mL), spiked with AFM_1 at a concentration of 0.5 ng/mL, heated to approximately 35–37°C, and then centrifuged at 4,000 rpm for 10 min. A 25-mL volume of milk sample was passed through the AFLAPREP® M column (Biopharm, Germany) by gravity flow to facilitate toxin binding. The column was washed with 20 mL of PBS at a flow rate of approximately 5 mL per min. Toxin was eluted from the

FIGURE 2 | Optimization of cDNA sequence, concentration, and reaction stability. **(A)** Optimization of cDNA by varying its length (cDNAs 1–7). Black bars represent fluorescence intensity before addition of AFM_1, and blue bars represent the fluorescence intensity after addition of 150 ng/mL AFM_1. **(B)** Fold increase in fluorescence (F/F_0) after addition of 150 ng/mL AFM_1. **(C)** Optimization of the aptamer:cDNA5 concentration ratio in the presence of 150 ng/mL AFM_1. **(D)** Study on the stability of AFM_1 fluorescence sensor after addition of 150 ng/mL AFM_1. Data represent means and standard deviations from three parallel experiments. Excitation/emission: $\lambda_{ex}/\lambda_{em} = 495/520$ nm. Reaction conditions: 10 nM AFM_1 aptamer and 20 nM cDNA in Tris-HCl buffer (10 mM, pH 7.0).

column with 1.25 mL of methanol:acetonitrile (40:60 v/v) at a flow rate of one drop per second; following elution, 1.25 mL of water was run through the column and collected in the same vial to yield a total volume of 2.5 mL, which was concentrated to 1 mL in a N1-Automatic Nitrogen Concentrator. After concentration, 1.5 mL Tris-HCl buffer was added. Subsequently, the fluorescence signal was determined. Five replicates of each sample were measured to evaluate the accuracy of the method.

RESULTS AND DISCUSSION

Design of the Aptamer-Based Fluorescence Quenching Platform

A schematic diagram of the fluorimetric design for AFM_1 detection is shown in **Figure 1A**. Theoretically, the sensor functionality was based on changes in the conformation of the aptamer after formation of the AFM_1/aptamer complex and the resultant change in fluorescence signal. In our sensor

system, AFM_1 aptamers were modified with FAM, and 7-mer cDNAs with the TAMRA quenching group. In the absence of AFM_1, the aptamers were hybridized with cDNA, resulting in quenching of their fluorescence due to the proximity of TAMRA-cDNA (Sharma et al., 2016). Upon the AFM_1 addition, a structural switch was induced in the AFM_1 aptamer, leading to formation of an AFM_1/aptamer complex (Seok et al., 2015). Changes in the structure of the aptamer caused the release of cDNA from the aptamer, and ultimately to the recovery of aptamer fluorescence. Therefore, the concentration of AFM_1 and the change in fluorescence intensity after the reaction were positively correlated, and the fluorescence change could be used to quantify the level of AFM_1. To confirm that AFM_1 could dissociate the aptamer–cDNA complex and restore the aptamer fluorescence, we added 150 ng/mL AFM_1 to 10 nM aptamer and 20 nM cDNA in Tris-HCl buffer. **Figure 1B** shows that fluorescence intensity increased at least 11-fold after the addition of AFM_1. Moreover, these findings confirm that the covalently labeled fluorophore
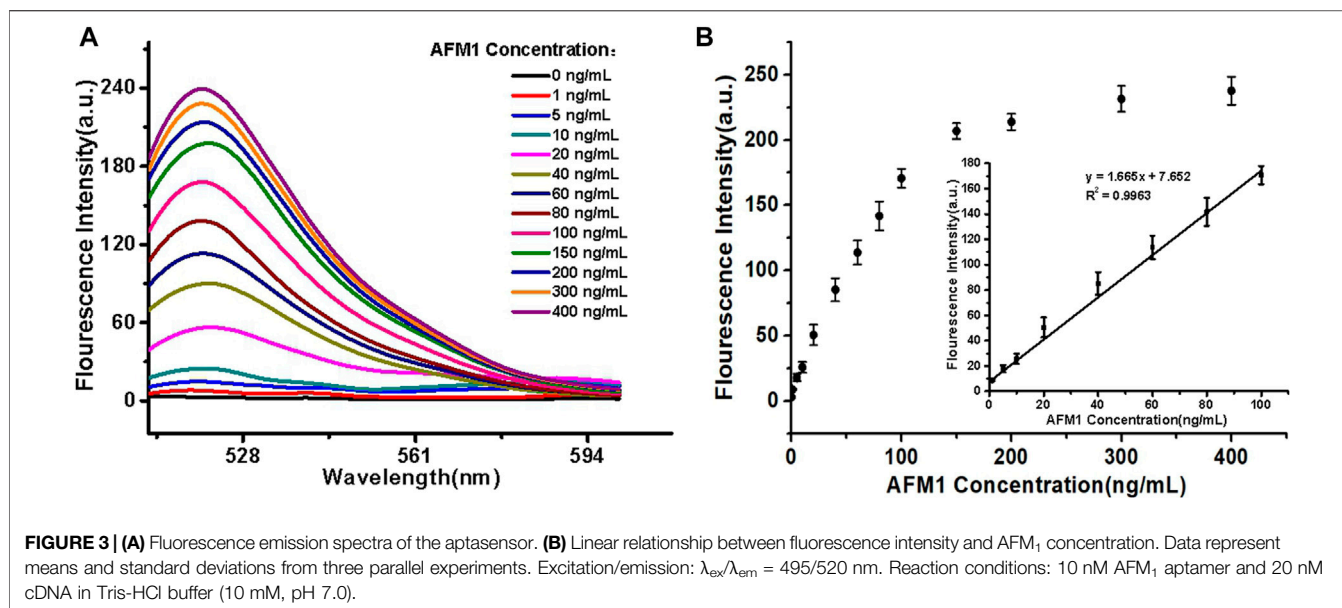
FIGURE 3 | (A) Fluorescence emission spectra of the aptasensor. **(B)** Linear relationship between fluorescence intensity and AFM_1 concentration. Data represent means and standard deviations from three parallel experiments. Excitation/emission: $\lambda_{ex}/\lambda_{em}$ = 495/520 nm. Reaction conditions: 10 nM AFM_1 aptamer and 20 nM cDNA in Tris-HCl buffer (10 mM, pH 7.0).

TABLE 1 | Comparison of the aptasensor with previously reported methods for detection of AFM_1.

Detection methods	Transduction principle	LOD	References
Instrumental methods	HPLC	6×10^{-3} ng/mL	Wang et al. (2012)
	SPE-UPLC–MS/MS	0.3×10^{-3} ng/mL	Wang and Li (2015)
Antibody-based methods	Impedimetric biosensor	1 ng/mL	Dinakaya et al. (2011)
	Enzyme immunoassay	5.0×10^{-3} ng/mL	Anfossi et al. (2008)
	Electrochemical immunosensors	1.0×10^{-6} ng/mL	Neagu et al. (2009)
Aptamer-based methods	Immunochromatographic assay	0.05 ng/mL	Wu et al. (2016)
	Label free polyaniline based aptasensor	1.98×10^{-3} ng/mL	Nguyen et al. (2013)
	Electrochemical impedance spectroscopy aptasensor	1.15×10^{-3} ng/mL	Istamboulia et al. (2016)
	RT-qPCR based aptasensor	0.03×10^{-3} ng/mL	Guo et al. (2016)
	Structure switching signaling aptamer assay	5.0×10^{-3} ng/mL	Sharma et al. (2016)
	Visual electrochemiluminescence biosensing	0.05 ng/mL	Khoshfetrat et al. (2017)
	Aptamer-based fluorescence-quenching assay	0.5 ng/mL	This work

(FAM) did not disrupt the original recognition properties of the AFM_1 aptamer.
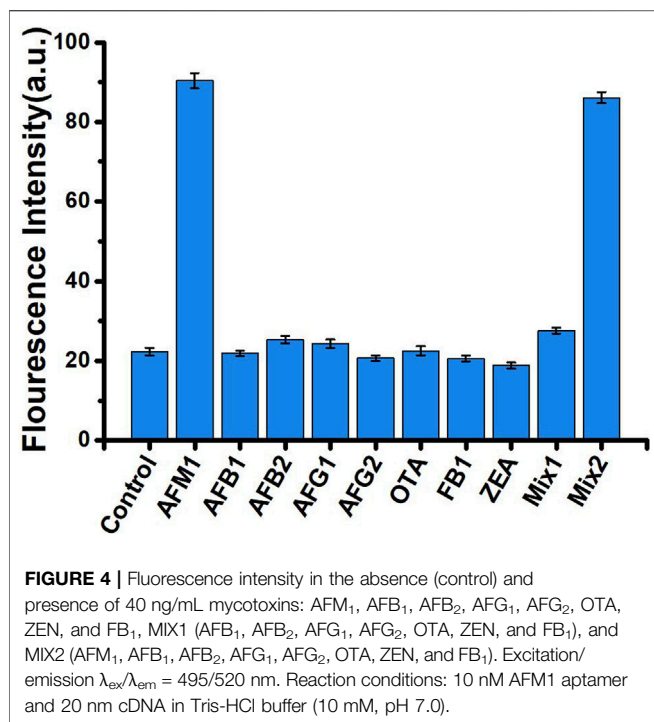
Optimization of the cDNA Sequence

Next, we optimized the number of cDNA bases to stabilize the aptamer/cDNA duplex and minimize background fluorescence in the absence of AFM_1. Accordingly, to optimize the sensor, we designed a series of complementary single-stranded DNA sequences. In this experiment, 10 nM AFM_1 aptamer was added to 20 nM of cDNAs 1–7, incubated for 5 min at 88°C, and then cooled to room temperature for 30 min to *prepare* add 150 ng/mL AFM_1. In **Figure 2A**, black columns represent fluorescence background after hybridization of AFM_1 aptamers with cDNA1–cDNA7 in the absence of AFM_1, and the blue columns represent fluorescence intensity after addition of 150 ng/mL AFM_1. The results revealed that cDNA5–7 could effectively quench AFM_1 aptamer fluorescence and decrease background signal in the absence of AFM_1. Due to the short lengths of cDNAs 1–4, hybridization with *AFM1* aptamer was unstable, resulting in a

higher background signal. When cDNA5 was hybridized with the aptamer, a significant increase in fluorescence (F/F_0) was observed after addition of AFM_1, whereas the longer cDNA sequences (cDNA6 and cDNA7) effectively quenched fluorescence (**Figure 2B**). This may be because of cDNA5, which contains more nucleotides than the shorter sequences, and could promote the formation of cDNA–aptamer duplexes while limiting AFM_1-induced structural transitions of the aptamer. Therefore, we considered cDNA5 to be the optimal single-stranded cDNA sequence, and used it in subsequent experiments.

Optimization of cDNA5 Concentration

To further improve the performance of the sensor, we optimized the cDNA5 concentration. In this experiment, 10 nM aptamer was mixed with cDNA5 at a molar ratio of 1:1, 1:2, 1:3, 1:4, or 1:5. Subsequently, 150 ng/mL AFM_1 was added, and the change in fluorescence signal was monitored. As shown in **Figure 2C**, the maximum fluorescence enhancement (F/F_0) was highest at a cDNA5 concentration of 20 nM. Thus, even at higher

FIGURE 4 | Fluorescence intensity in the absence (control) and presence of 40 ng/mL mycotoxins: AFM$_1$, AFB$_1$, AFB$_2$, AFG$_1$, AFG$_2$, OTA, ZEN, and FB$_1$, MIX1 (AFB$_1$, AFB$_2$, AFG$_1$, AFG$_2$, OTA, ZEN, and FB$_1$), and MIX2 (AFM$_1$, AFB$_1$, AFB$_2$, AFG$_1$, AFG$_2$, OTA, ZEN, and FB$_1$). Excitation/emission $\lambda_{ex}/\lambda_{em}$ = 495/520 nm. Reaction conditions: 10 nM AFM1 aptamer and 20 nm cDNA in Tris-HCl buffer (10 mM, pH 7.0).

concentrations of cDNA5, the formation of stable cDNA5/aptamer duplexes generated low background fluorescence. When the concentration was higher than 20 nM (twice the AFM$_1$ aptamer concentration), it affected the optimal AFM$_1$ interaction, thereby limiting the increase in fluorescence. Accordingly, for subsequent sensing experiments, we determined the optimum concentration of cDNA5 to be 20 nM.

Stability of the Reaction

To generate the desired fluorescence signal response, we analyzed the reaction time of AFM$_1$ with the aptamer. As shown in **Figure 2D**, the aptamer and AFM$_1$ combined rapidly: the concentration of AFM$_1$ was detected at 1 min, and the fluorescence value remained stable for up to 30 min. Therefore, the fluorescence sensor had acceptable stability. We determined that a reaction time of 15 min was optimal for this assay.

Quantitative Determination of AFM$_1$

For all experimental conditions, the excitation and emission wavelengths were 495 and 520 nm, respectively. The sensor

detected different concentrations of AFM$_1$ (0, 1, 5, 10, 20, 40, 60, 80, 100, 150, 200, 300, and 400 ng/mL) with corresponding fluorescence signal intensities. As the concentrations of AFM$_1$ in the reaction system were increased, the released amounts of cDNA were also increased, leading to quenching groups located away from the fluorophore and an increase in fluorescence value. As shown in **Figure 3A**, the fluorescence intensity increased with the increased AFM$_1$ concentration, and fluorescence intensity was maximal at an AFM$_1$ concentration of 150 ng/mL. The concentration of AFM$_1$ was linear in the range of 1–100 ng/mL (**Figure 3B**), and the linear regression equation was F = 1.665C + 7.652 (R^2 = 0.9963), in which F denotes the fluorescence intensity and C denotes the AFM$_1$ concentration. The limit of detection (LOD) was 0.5 ng/mL with a signal-to-noize ratio of 3 (S/N = 3). Although the proposed detection method gave lower or comparable detection limits compared to other instruments and rapid screening methods (**Table 1**), this sensor provides a novel, simple, inexpensive and portable idea for detecting AFM$_1$.

Specificity of the Assay

Specificity is an important measure of an aptamer sensor. To evaluate the specificity of our aptasensors, we selected seven mycotoxins with structural similarities to AFM$_1$ (AFB$_1$, AFB$_2$, AFG$_1$, AFG$_2$, OTA, ZEN, and FB$_1$) as controls. Each of these mycotoxins was examined at the same concentration (40 ng/mL) under the same experimental conditions used for AFM$_1$. As shown in **Figure 4**, neither the individual mycotoxins nor a mixture of all compounds other than AFM$_1$ (MIX1) significantly altered fluorescence relative to the no-mycotoxin control ($p >$ 0.05). A mixture of all toxins including AFM$_1$ (MIX2) had a slightly weaker effect than AFM$_1$ individually, but the difference was not significant. These results confirmed that this fluorescence biosensor did not react mycotoxins other than AFM$_1$, indicating that the method was highly specific for detection of AFM$_1$.

Milk Sample Analysis

To further verify practicality and feasibility, we applied our method to the detection of AFM$_1$ in two different brands of spiked milk samples. Four different concentrations of AFM$_1$ (0.5, 5, 10, and 20 ng/mL) were spiked into milk samples, and sample preparation was performed as described in *Real Sample Analysis* section, and five replicates were tested for each concentration. As shown in **Table 2**, the recoveries of the spiked milk samples were in the range 93.4–101.3% (n = 5), respectively, indicating that the

TABLE 2 | Determination of AFM$_1$ spiked into milk samples (n = 5).

Spiked concentration (ng/mL)	Detected concentrations (ng/mL)[a]	RSD (%)	Recovery (%)
0.5	0.47	4.8	93.4
5	5.1	3.6	101.3
10	9.7	2.9	97.5
20	19.4	2.6	97

[a]*Mean of five replicates.*

aptasensor could be used as a quantitative method for AFM_1 detection in real milk samples.

CONCLUSION

In summary, we successfully developed an aptamer-based fluorescence quenching assay for direct detection of AFM_1 and confirmed the feasibility of this method for use on milk samples. Under optimal conditions, the aptasensor exhibited linear increases in fluorescence intensity in response to increasing concentrations of AFM_1 from 1 to 100 ng/mL, with a LOD of 0.5 ng/mL. This assay was highly specific for AFM_1 and did not detect other toxins with similar structures, including AFB_1, AFB_2, AFG_1, AFG_2, OTA, ZEA, and FB_1. Recoveries of AFM_1 from spiked milk samples were in the range of 93.4–101.3% (n = 5). This assay offers a novel, simple, rapid, specific, convenient, and cost-effective approach for detection of AFM_1, and provides a means of quantitatively determining mycotoxins in dairy products. In future studies, the method of target recycling of signal amplification to improve the sensitivity of aptamer-based fluorescence biosensors will be conducted. At the same time, we clearly understand that the interference from the milk matrix to optical measurement is typically very challenging. Therefore, strict sample pre-treatment was required for eliminating the effects of the background due to milk on the aptasensor detection, which is also the challenge and future development trend of nucleic acid aptamer detection of mycotoxins.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENTS

All participants were informed about the aim of the study and gave written consent.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

FUNDING

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