ORIGINAL PAPER

Detection of ochratoxin A using a "turn-on" fluorescence assay based on guanine quenching of the aptamer

Limin Guo[1] · Yun Li[1] · Shichao Gao[1] · Lei Ren[1]

Abstract

Ochratoxin A (OTA) is a common mycotoxin with high carcinogenicity; therefore, it is crucial to establish a simple, rapid, and sensitive method for its detection. In this study, we developed a "turn-on" fluorescence assay for detecting OTA based on guanine quenching of the aptamer. The method uses fluorescein (FAM) fluorophore to label the complementary strand of the OTA aptamer, Fc-DNA. In the absence of OTA, the Fc-DNA hybridizes with the aptamer to form a double strand. Due to the occurrence of photo-induced electron transfer (PET), the FAM fluorescence signal is quenched as the FAM on the Fc-DNA approaches the guanine of the aptamer at the 5′ end. When OTA is present, the aptamer binds to it and thus, is unable to hybridize with Fc-DNA to form a double strand; the FAM fluorescence signal is restored as FAM moves away from the guanine of the aptamer. The assay achieved OTA detection at a detection limit of 28.4 nM. The application of the original guanine of the aptamer as the quenching agent helps avoid the complex designing and labeling of the aptamer, which ensures the high affinity of the aptamer for OTA. Meanwhile, this "turn-on" detection mode helps avoid potential false-positive results as in the "turn-off" mode and improves the assay's sensitivity. Additionally, the method has good selectivity and can be used to detect OTA in traditional Chinese medicine. This method provides a simple, low-cost, and rapid method for OTA detection.

Keywords Aptamer · Fluorescence · Guanine quenching · OTA · Herbal medicine

Introduction

Herbal medicines have been used in the treatment of human diseases for millennia as important components of various traditional medicine systems. The World Health Organization reported that 70–80% of the global population uses herbal medicines for primary healthcare [1]. With the rapid outbreak of COVID-19 in 2019, herbal medicines have been playing an active role in combating the epidemic [2, 3]. However, there are concerns regarding the contaminants present in herbal medicines, with ochratoxin A (OTA) being a common contaminant.

As a secondary fungal metabolite, OTA is one of the most widely observed contaminants and a key component of mycotoxins. OTA exhibits nephrotoxicity and immunotoxicity, can cause renal tumors in animals [4] and is considered a potential carcinogen (group 2B). In particular, OTA is ubiquitous in many herbal medicines and may be produced during the various stages of the production cycle of herbal medicines, including their growth, harvest, storage, and processing. Therefore, monitoring OTA is of great significance for the safety of herbal medicines.

Conventionally, OTA is detected using chromatographic methods, such as thin-layer chromatography [5], high-performance liquid chromatography (HPLC) [6], HPLC coupled to fluorescence and mass spectrometry [7, 8], and enzyme-linked immunosorbent assay (ELISA) [9]. However, these methods are often expensive, complex, and require experienced personnel. In addition, ELISA results can often be false positives or -negatives. More importantly, OTA is a small-molecule biological toxin with low immunogenicity, limiting the application of immunosensors. This obstacle; however, can be overcome by using an aptamer-based biosensor for OTA detection.

✉ Limin Guo
    619545401@qq.com

1   College of traditional Chinese medicine and food engineering, Shanxi University of Chinese Medicine, 121 University Street, Yuci, Jinzhong 030619, People's Republic of China

Aptamers, which are a type of synthetic antibodies, are DNA or RNA fragments that can selectively interact with a specific target with strong affinity [10–12]. They can be obtained by in vitro selection, making them independent of the target antigenicity. Therefore, aptamers are highly suited for use as recognition modules for low-immunogenicity small molecules, such as OTA. They show superior characteristics, such as high stability, easy use, and high binding affinity and specificity compared to conventional methods. Since the discovery of the specific aptamer for OTA [13], various aptamer-based sensors have been described as alternatives to classical analytical methods [14–18]. Among the different types of detection methods available, fluorescence-based assays are particularly attractive and popular, mainly owing to their unique advantages, such as ease of manipulation, high sensitivity, feasible quantification, fast response, and potential for high-throughput analysis.

It is a common fluorescence assay that the fluorescence intensity is changed by quenching the fluorescence of fluorophore with a quenching agent. This method typically has two detection modes: "turn-on" and "turn-off" for signal enhancement and attenuation, respectively. "Turn-off" detection mode is usually followed by the addition of the target, and the fluorescence signal is quenched due to the fluorophore's proximity to the quenching group. This mode has a high detection background, low detection sensitivity, and may produce false-positive results. The "turn-on" mode [19, 20] has received widespread attention due to its high sensitivity and selectivity. Some methods in "turn-on" mode need to label the quenching agent, such as the black hole quencher and 5-(dimethylamino) naphthalene-1-sulfonic acid (DANSYL), as well as the fluorophore on the aptamer [21]. This double-labeled probe may affect the binding of the aptamer to the target. Label-free methods mostly use nanomaterials, such as carbon nanotubes, graphene, and nanometals, as quenching agents [22]. But the application of nanomaterials in food detection suffers from disadvantages including water stability and interference of other analytes, which would also incur higher production costs. For example, using single-walled carbon nanohorns (SWCNHs) as fluorescence quencher, Guo et al. [23]. proposed a fluorescent aptasensor for OTA detection, this method was label-free and "turn-on" detection strategy, However, the defects of nanomaterial itself restricts its wide application.

Some studies reported label-free methods for OTA detection based on the structural changes of aptamer binding to OTA. Delaney E et al. [24]. reported that OTA binding to aptamer involves π-stacking interactions that lead to GQ (antiparallel G-quadruplex) -to-OTA energy transfer (ET), resulting in enhanced fluorescence signal in the aptamer-OTA complex compared to free OTA alone. This method, however, cannot distinguish OTA from other ochratoxin (such as OTB). Liu et al. [25]. found that under the induction of OTA, the aptamer structure was transformed into G-quadruplex, and the zinc (II) -protoporphyrin IX probe specifically bound to G-quadruplex to produce strong fluorescence. However, the narrow detection range of this method (0.1–1.2 nM) limits its application in practical samples.

Photo-induced electron transfer (PET) is the process of transferring energy from a molecule excited by light (donor) to another molecule in the ground state (acceptor). Guanine is an excellent acceptor, which can undergo PET with a nearby fluorophore, thus its quenching fluorescence. Based on the principle of PET detection, guanine is used as a fluorescence quenching agent, and several methods focus on the detection of heavy metals, such as Hg^{2+} and Pb^{2+} [26, 27]. These assays label the fluorophore at one end of the aptamer, add a few guanines at the other end, or design the aptamer as a hairpin structure. After the target is added, the aptamer configuration changes, with the two ends getting closer together and the FAM fluorescence signal being quenched by guanine. Most of these methods are conducted in the "turn-off" detection mode, and the aptamer is labeled and modified multiple times [28, 29]. Some studies report the use of the same detection principle for OTA detection. Zhao et al. labeled FAM on the OTA aptamer, and added guanine to the 3′ end of its complementary strand to quench FAM [30]. Hitabatuma et al. designed a molecular beacon with the fluorophore FAM and the DANSYL quencher labeled on its 3′ and 5′ ends, respectively [31]. The addition of OTA separates the 3′ and 5′ ends of the molecular beacon further away, and the FAM fluorescence signal was restored. These two detection methods were in the "turn-on" detection mode, but they both designed and labelled the aptamer, which may affect the binding affinity of OTA-aptamer and OTA and also increases the detection cost.

The application of the original guanine of the aptamer as a fluorescence-quenching agent helps avoid the tedious quenching agent labeling process of the DNA strand and does not need the addition of expensive nanomaterials as quenching agents, providing a novel fluorescence detection mode for quantitative determination. However, there are few reports on this method, and limitations, such as the small fluorescence recovery value and complex sample pretreatment need to be overcome [32]. Therefore, the development of a suitable method for OTA detection is still a tremendous challenge.

In the present study, we developed a "turn-on" fluorescence method for OTA determination based on the PET principle, with the aptamer as the recognition element and the guanine of the aptamer as the quenching agent. We designed an Fc-DNA of the OTA aptamer and labeled the FAM fluorophore at its 3′ end. In the absence of OTA, the aptamer hybridizes with Fc-DNA to form a double strand, and the FAM fluorescence signal is instantly quenched by

the guanine at the 5′ end of the aptamer through PET. In the presence of OTA, the aptamer preferentially binds to it instead of hybridizing with Fc-DNA to form a double strand, and the FAM fluorescence signal is restored as FAM moves away from the guanine of the aptamer. In this Scheme, we examined in detail the effect of guanine at different positions of the aptamer on the fluorescence signal, selected the optimal length of Fc-DNA, and optimized other experimental conditions, including ionic strength, aptamer concentration, and pH. As this method uses the original guanine of the aptamer as the quenching agent without any modification or labeling, the affinity of the aptamer for OTA is guaranteed. Additionally, this "turn-on" detection mode helps avoid potential false-positive results as in the turn-off mode and improves the assay's sensitivity. This assay has been used to detect OTA in several herbal medicines and has yielded satisfactory results.

Experimental

Reagents and materials used

OTA, ochratoxin B (OTB), n-acetyl-L-phenylalanine (NAP), warfarin (WF), and 7-amino-4-methylcoumarin (AMC) were obtained from Sigma-Aldrich (St. Louis, MO, USA). aflatoxin B2 (AFB2), and zearalenone (ZEA) were obtained from Shanghai Biotechnology Co. Ltd. (Shanghai, China). *Platycodon grandiflorum, Areca catechu* and *hibiscus bark* were obtained commercially (Sichuan, China). All the oligonucleotides (Table 1) were synthesized and purified by Shanghai Biotechnology Co. Ltd. (Shanghai, China). The buffer solution used was 20 mM Tris–HCl containing 5 mM $MgCl_2$ and 10 mM NaCl and had a pH of 8.0. Ultrapure water (UPR-11-20L, ULUPURE, China) was used during all the experiments. All the fluorescence measurements were performed on a LS 45 Luminescence Spectrometer (Perkin Elmer Instruments, USA) with excitation at 496 nm and emission at 518 nm. The slit width for excitation and emission was set at 10 nm.

Experimental procedures for detecting OTA

All steps of this "turn-on" fluorescence method, which used guanine of the aptamer as the quenching agent, were performed at room temperature. First, 40 µL of 450 nM aptamer and 40 µL of OTA solution at different concentrations were mixed using a mixer for 40 min. Then 40 µL of 240 nM Fc-DNA was added and mixed for 30 min. Finally, the fluorescence signal of the solution was measured.

Specificity analysis

To test the specificity of the "turn-on" assay based on guanine quenching of the aptamer for OTA, Three OTA analogs (NAP, AMC, and WF) and three mycotoxins (OTB, AFB2, and ZEA) that may coexist with OTA were tested at different concentrations (80, 300, 600 nM). The detection steps were the same as those mentioned above.

Detection of OTA in herbal medicines

To verify the feasibility of the "turn-on" assay based on guanine quenching of the aptamer for OTA detection in actual herbal medicine samples, *Platycodon grandiflorum*, *Areca catechu*, and *hibiscus bark* were used for the assay. First, 5 g of each sample was extracted with 20 mL of ultrapure water by heating and boiling for 10 min and subsequent centrifugation at 10,000 rpm for 15 min at room temperature. Then, the extracts were diluted 20-fold with a buffer solution to minimize the influence of the complex matrix. Finally, OTA was added to the diluted extracts at different concentrations, and the detection was performed.

Results and discussion

Working principle of the OTA sensing system

Scheme 1 shows the detection principle behind the OTA sensing system. In the absence of OTA, the aptamer and Fc-DNA hybridize to form a double strand. Simultaneously, the FAM fluorescence signal is quenched when PET occurs as the FAM on the Fc-DNA approaches the guanine of the aptamer at the 5′ end. When OTA is added to the solution, the aptamer preferentially binds to it and cannot hybridize with Fc-DNA due to the strong affinity between the aptamer and OTA. FAM is far from the aptamer guanine, which is

Table 1 List of oligonucleotide sequences

Name	Sequences
Aptamer	5′-GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA-3′
Fc5-1G-DNA	5′-CG ATC-FAM -3′
Fc6-1G-DNA	5′-CCG ATC-FAM-3′
Fc7-1G-DNA	5′-C CCG ATC-FAM-3′
Fc8-1G-DNA	5′-AC CCG ATC-FAM-3′
Fc9-1G-DNA	5′-CAC CCG ATC-FAM-3′
Fc7-6G-DNA	5′-C CCA CAC -FAM -3′
Fc7-15G-DNA	5′-FAM-CAC CCA C-3′
Fc7-25G-DNA	5′-G ATG CTC-FAM -3′
Fc7-33G-DNA	5′-FAM-CG ATG CT-3′

Scheme 1 Schematic illustration of the "turn-on" assay based on guanine quenching of the aptamer
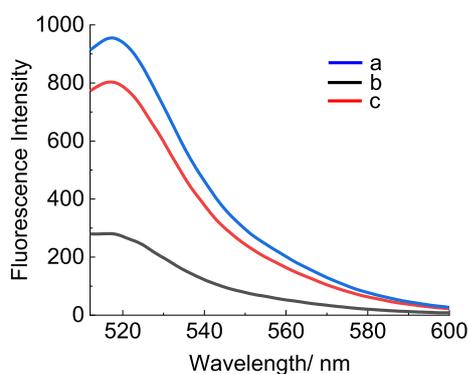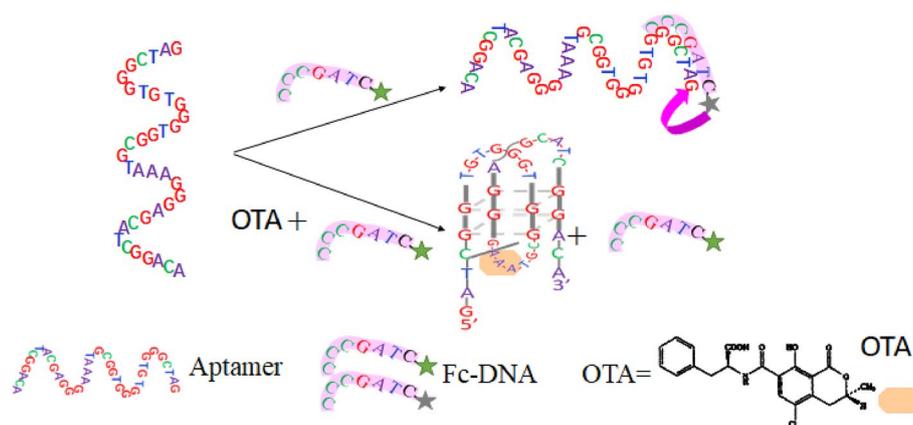
Fig. 1 Feasibility of the "turn-on" assay based on guanine quenching of the aptamer. Line a: the fluorescence intensity of Fc-DNA, line b: the fluorescence intensity of Fc-DNA/aptamer, line c: the fluorescence intensity of Fc-DNA/aptamer/OTA.Experimental conditions: Fc-DNA (80 nM), aptamer (150 nM), OTA (600 nM)

why its fluorescence signal remains unquenched. The higher the OTA concentration in the solution, the lesser the aptamer hybridizes with Fc-DNA. The farther the FAM is from the aptamer, the stronger the fluorescence signal of the system. The enhanced ratio of FAM fluorescence signal $\Delta F/F_0$ enables the quantitative detection of OTA ($\Delta F = F - F_0$, F is the fluorescence signal of the sample with OTA and F_0 is the fluorescence signal of the sample without OTA).

Feasibility test

To test whether this "turn-on" fluorescence method, which employs guanine of the aptamer as a quenching agent, can be used to detect OTA, we first performed feasibility experiments. FAM emitted a strong fluorescence signal when only Fc-DNA was present in the solution (Fig. 1a). When an aptamer was added, it hybridized with Fc-DNA to form a double strand. The FAM fluorescence signal was quenched when PET occurred as FAM approached the guanine of the aptamer, and the fluorescence signal dropped sharply (Fig. 1b). When OTA was present in the solution, the aptamer bound to it, the FAM on Fc-DNA moved away from the aptamer, and the fluorescence signal of the system was restored (Fig. 1c). This result showed that the presence of OTA reduced the hybridization of the aptamer with Fc-DNA, indicating that the method can be used for the quantitative determination of OTA.

Optimization of the experimental conditions

To improve the sensitivity of the method, we optimized the experimental conditions, including the guanine position in the aptamer as a quenching agent, the length of the complementary strand, the concentration of Mg^{2+} and the aptamer, and the effect of pH.

Fc-DNA optimization

(1) Optimization of the position. In this method, the guanines in the aptamer quenched the FAM on Fc-DNA, and the higher the quenching efficiency, the lower the detection background of the system and higher the sensitivity of the method. As the OTA aptamer is a G-rich DNA sequence, there are several guanines on the aptamer, and the different positions of the nucleotide may affect their FAM fluorescence quenching abilities; therefore, selecting the appropriate position of the guanines is especially crucial. Five Fc-DNAs were designed in the experiment. In each of them, FAM was placed near five different guanines on the aptamer sequence (including the guanine at the 5' end, three guanines in the middle of the aptamer sequence, and the guanine closest to the 3′ end). As indicated in Fig. 2, only guanines at the 5′ end (Fc7-1G-DNA) showed the best response. It is reported [33] that when the guanine was used as the quenching agent, the more exposed the guanine was, the more efficient the quenching was. Compared with other guanine in the aptamer, the guanine of the aptamer at the 5' end was the first base, which was
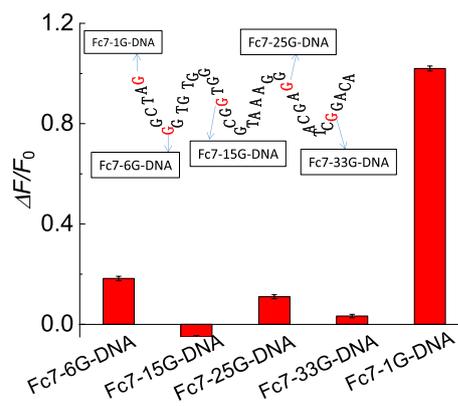
Fig. 2 Effect of Fc-DNA quenched by different guanines in the aptamer. Experimental conditions: Fc-DNA (80 nM), aptamer (150 nM), OTA (300 nM). Inset showed the sequence of aptamer with the locations of the five guanines

in a more exposed environment. This experiment showed that its fluorescence quenching is more efficient, which is consistent with literature reports. The fluorescence of FAM in the complementary strand Fc7-1G-DNA is quenched by the guanine of the aptamer at the 5' end, therefore, Fc7-1G-DNA was selected for subsequent experiments.

(2) Optimization of the complementary strand length. In this experiment, the Fc-DNA complementary strand and OTA competed for binding to the aptamer, and the length of the Fc-DNA strand had a remarkable effect on the fluorescence signal. Five different Fc-DNA base lengths were considered in the experiment. The results are displayed in Fig. S1. As the number of bases of the Fc-DNA increased, its ability to hybridize with the aptamer gradually increased and the fluorescence quenching of guanines on FAM became more effective (F_0 gradually decreases). However, after OTA was added, the higher the number of bases, the more difficult it was for the double strand formed through hybridization of Fc-DNA and aptamer to dissociate, and the less the effective recovery of the FAM fluorescence signal (the lower the F value). Either too high F_0 value or too low F value would reduce the fluorescence enhancement ratio $\Delta F/F_0$, resulting in decreased sensitivity and a narrower detection range. In comparison among the five base lengths considered, the best response was reported for Fc7-1G-DNA containing seven bases.

Optimization of the aptamer concentration

As the aptamer is not only a recognition element for OTA but also a quenching agent for the FAM fluorophore, its concentration has a considerable effect on the signal. In this experiment, we examined different aptamer concentrations. As shown in Fig. S2, with the increase in aptamer concentration, the $\Delta F/F_0$ value increased sharply

and reached its peak at 150 nM, indicating that the guanine of the aptamer saturated the FAM quenching at this point. As we increased the aptamer concentration beyond this point, a slight decrease in $\Delta F/F_0$ was observed; thus, we selected 150 nM as the aptamer concentration for subsequent experiments.

pH optimization

A series of Tris–HCl buffers of different pH (7.0, 7.5, 8.0, 8.5, 9.0, and 9.5) containing 5 mM Mg^{2+} and 10 mM Na^+ was prepared, and the effect of the pH on $\Delta F/F_0$ was determined (Fig. S3). The results showed that $\Delta F/F_0$ increased with the increase in the pH between 7.0 and 8.0, reaching its peak at pH 8.0. Moreover, when the pH was increased to values higher than 8.0, $\Delta F/F_0$ decreased remarkably. Thus, for the subsequent experiments, a binding buffer solution at pH 8.0 was used.

Optimization of the Mg^{2+} concentration

Metal ions have a considerable effect on aptamer stability and improve the binding affinity between aptamers and their targets [34, 35]. To elucidate the effect of ionic strength on OTA detection, the optimal Mg^{2+} concentration was determined. As shown in Fig. S4, the initial fluorescence without Mg^{2+} was low. However, $\Delta F/F_0$ continued to increase for Mg^{2+} concentrations between 2–5 mM but decreased with an increase beyond this range. Therefore, 5 mM Mg^{2+} was selected as the ideal concentration for subsequent experiments.

OTA detection performance

The sensitivity of the as-built fluorescence assay for OTA detection was explored by analyzing different OTA concentrations under the optimized experimental conditions. Figure 3a shows that the method was highly dependent on the concentration of the target OTA. A gradual increase in the fluorescence intensity was observed as the OTA concentration increased from 0 to 750 nM. This increase could be ascribed to the specific recognition of OTA by the aptamer, which prevented the hybridization of the Fc-DNA with it, thus restoring the FAM fluorescence signal. The plot of the changes in the relative fluorescence intensity of the system, $\Delta F/F_0$ with OTA concentration within the investigated range is shown in Fig. 3b. The linear regression equation is as follows: $Y = 0.00342X + 0.0015$, with $R^2 = 0.994$, where Y and X represent $\Delta F/F_0$ and OTA concentration, respectively. The linear correlation between $\Delta F/F_0$ and OTA concentration was observed over the range 30–600 nM. The detection limit (LOD) determined based on 3 times the standard deviation of the blank was
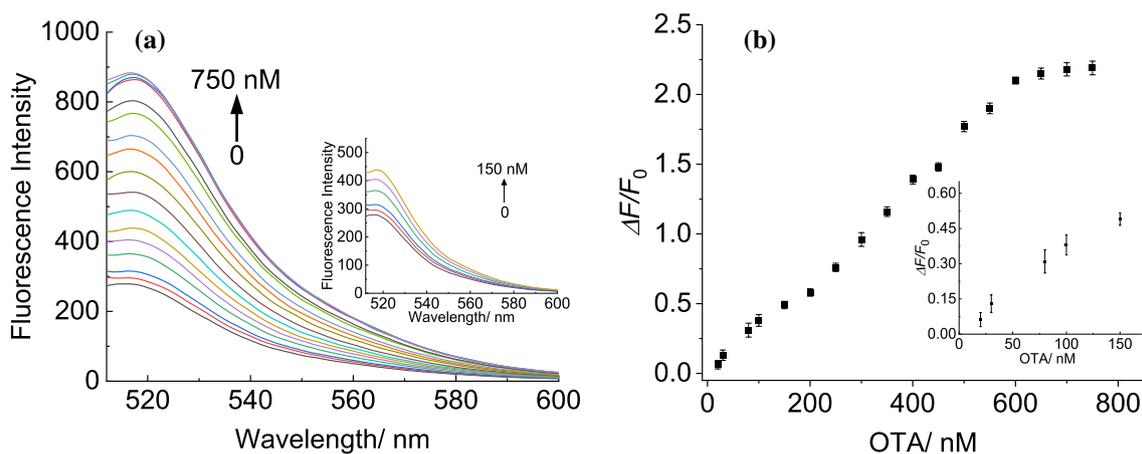
Fig. 3 Quantification of OTA in the "turn-on" assay based on guanine quenching of the aptamer. **A** Fluorescence spectra of Fc-DNA/aptamer/OTA with the concentration of OTA (0–750 nM). Inset showed the enlarged low concentrations range (0–150 nM);

B Calibration curve of the $\Delta F/F_0$ vs. the concentrations of OTA: 0–750 nM (Inset showed the calibration curve of the $\Delta F/F_0$ vs. the concentrations of OTA in the range of 20–150 nM). Error bars were obtained from four independent experimental data

28.4 nM. Table S1 lists the results of some recently reported quencher-based assays for OTA [20, 23, 36–39]. The LOD of the method developed in this study is comparable to that of previous reports. More importantly, the proposed system is simple and sensitive owing to its "turn-on" and label-free design.

Specificity test

Aptamers are primarily obtained through a method called systematic evolution of ligands by exponential enrichment. This method ensures the specificity of the aptamers towards the target molecules. Three OTA analogs (NAP, AMC, and WF) and three mycotoxins (OTB, AFB2, and ZEA) that may coexist with OTA were tested at different concentrations. The results are presented in Fig. 4. We observed that only OTA produced a remarkable fluorescence signal, while the structural OTA analogs and mycotoxins showed negligible changes in signal intensity. In addition, all the interference components resulted in signals similar to those of the blank control for all three concentrations investigated (80, 300, and 600 nM). The high selectivity of the proposed method can be ascribed to the high selectivity of the aptamer for OTA.

Detecting OTA in herbal medicine samples

To further evaluate the practical application of the developed method, OTA was detected in herbal medicine matrices using the "turn-on" assay based on guanine quenching of the aptamer. Diluted (20×) extract samples of *Platycodon grandiflorum*, *Areca catechu*, and *hibiscus bark* were spiked with OTA at different concentrations (20, 50, 100, 300 and 600 nM) and the effect was observed. As shown in Table S2,
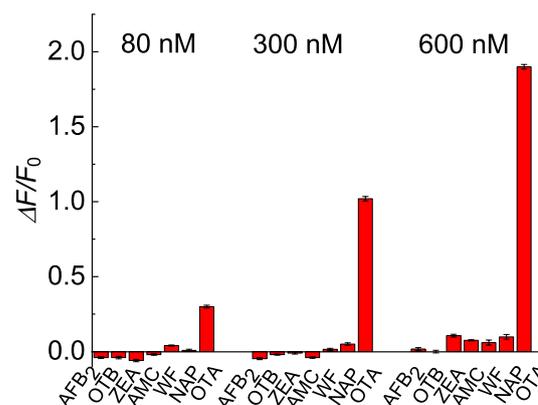
Fig. 4 Selectivity test for OTA compared to other mycotoxins and OTA analogs. Experimental conditions: Fc7-1G-DNA (80 nM), aptamer (150 nM)

the recovery ratio of the OTA added was estimated in the range of 89.2–114.6%. The homogeneous OTA detection in the herbal medicine samples using the proposed assay was simpler than that of conventional methods, such as HPLC and ELISA. In addition, the proposed method does not require time-consuming sample pretreatments and separation processes but only simple extraction and dilution.

Conclusions

Using guanine as the quenching agent and an aptamer as the recognition element, we developed a "turn-on" fluorescence method to detect OTA. In the absence of OTA, Fc-DNA hybridized with the aptamer to form a double strand, and

the fluorescence was quenched as FAM approached the guanine of the aptamer. In the presence of OTA, the aptamer remained preferentially bound to it, and the fluorescence was restored as the FAM moved away from guanine. The detection limit of the method was as low as 28.4 nM, and the linear range was 30–600 nM. The method showed satisfactory selectivity, and can be used for OTA detection in herbal medicine. It provided a new route for the qualitative estimation of herbal medicines.

Declarations

Conflicts of interest We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work; there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

References

1. O. Akerele, World Health Forum **14**, 390 (1993)
2. D.H. Zhang, K.L. Wu, X. Zhang, S.Q. Deng, B. Peng, J. Integr. Med. **18**, 152 (2020)
3. Z.B. Shao, Y.X. Zhu, S.H. Liu, K.J. Jiang, Q. Wu, J.Y. Shen, W.W. Liu, Chin Trad Herb Drugs **51**, 1153 (2020)
4. L. Caldeirão, J. Sousa, L.C.G. Nunes, H.T. Godoy, J.O. Fernandes, S.C. Cunha, Food Res. Int. **144**, 110322 (2021)
5. AE Ahmed, MM Al-Kahtani, EM EI-Diasty, AS Ahmed, H Saber, AM Abbas, HM Diab, MA Alshehri, AA Elmansi, MA Hussein. 2020 J. Pure Appl. Microbio. **14**:215
6. Z. Zareshahrabadi, R. Bahmyari, H. Nouraei, H. Khodadadi, J. Food Quality. **2**, 1 (2020)
7. M. Dhanshetty, K. Banerjee, Simultaneous. J. AOAC Int. **102**, 1666 (2019)
8. Z.Q. Zhang, Z.C. Fan, D.X. Nie, Z.H. Zhao, Z. Han, Molecules **24**, 2823 (2019)
9. Z.C. Sun, X.R. Wang, Z.W. Tang, Q. Chen, X. Liu, Ecotoxicol Environ Saf. **171**, 382 (2019)
10. C. Tuerk, L. Gold, Science **249**, 506 (1990)
11. D.L. Robertson, G.F. Joyce, Nature **344**, 467 (1990)
12. A.D. Ellington, J.W. Szostak, Nature **346**, 818 (1990)
13. J.A. Cruz-Aguado, G. Penner, Anal. Chem. **80**, 8853 (2008)
14. F.J. Zheng, W. Ke, L.X. Shi, H. Liu, Y. Zhao, Anal. Chem. **91**, 11812 (2019)
15. E. Moez, D. Noel, S. Brice, G. Benjamin, A. Pascaline, M. Didier, Food Chem. **310**, 125851 (2020)
16. Y. Yang, Y.A. Yin, S. Wang, Y.Y. Dong, Anal Chim Acta. **1155**, 338345 (2021)
17. L.X. Lu, W. Yuan, Q. Xiong, M.H. Wang, Y.J. Liu, M. Cao, X.H. Xiong, Anal Chim Acta. **1141**, 83 (2021)
18. F.Y. Tian, J. Zhou, B.N. Jiao, Y. He, Nanoscale **11**, 9547 (2019)
19. M. Lian, B. Xu, L.J. Liu, W.J. Tian, Chem. Res. Chim. Univ. **34**, 363 (2018)
20. L. Lv, C.B. Cui, C.Y. Liang, W.R. Quan, S.H. Wang, Z.J. Guo, Food Control **60**, 296 (2016)
21. S.M. Sanzani, M. Reverberi, C. Fanelli, A. Ippolito, Toxins **7**, 812 (2015)
22. L. Ma, B. Xu, L.J. Liu, W.J. Tian, Chem. Res. Chin. Univ. **34**, 363 (2018)
23. Z.J. Guo, J. Tian, C.B. Cui, Y. Wang, H.H. Yang, M. Yuan, H.S. Yu, Food Control **123**, 107741 (2021)
24. D.E. Armstrong-Price, P.S. Deore, R.A. Manderville, J. Agric. Food Chem. **68**, 2249 (2020)
25. F. Liu, A.L. Ding, J.S. Zheng, J.C. Chen, B. Wang, Sensors **18**, 1769 (2018)
26. H.S. Liu, L.B. Ma, C.B. Ma, J.Y. Du, M.L. Wang, K.M. Wang, Sensors **16**, 1945 (2016)
27. W.H. Wang, Y. Jin, Y.N. Zhao, X.F. Yue, C.X. Zhao, Biosens. Bioelectron. **41**, 137 (2013)
28. L. Wang, J.Q. Tian, H.L. Li, Y.W. Zhang, X.P. Sun, Analyst **136**, 891 (2011)
29. L. Wang, J.Q. Tian, H.L. Li, Y.W. Zhang, J. Fluoresc. **21**, 1049 (2011)
30. H. Zhao, X.Y. Xiang, M.J. Chen, C.B. Ma, Toxins **11**, 65 (2019)
31. A. Hitabatuma, Y.H. Pang, L.H. Yu, X.F. Shen, Food Chem. **342**, 128303 (2021)
32. Y. Qu, Y.Q. Zhang, G. Xiao, C. Yang, Chinese. J. Anal. Chem. **48**, 1409 (2020)
33. P. Qu, X.D. Chen, X.X. Zhou, X. Li, X.S. Zhao, Sci. China. Chem. **52**, 1653 (2009)
34. Q. Zhao, Q. Lv, H.L. Wang, Anal Chem. **86**, 1238 (2014)
35. Y.L. Zhang, W.H. Chen, X.T. Dong, H. Fan, X.H. Wang, L.J. Bian, Sens Actuators B Chem. **261**, 58 (2018)
36. A. Sharma, A. Hayat, R.K. Mishra, G. Catanante, S. Bhand, J.L. Marty, Toxins **7**, 3771 (2015)
37. X. Lv, Y.F. Zhang, G.F. Liu, L.Y. Du, S.H. Wang, RSC Adv. **7**, 16290 (2017)
38. L.F. Sheng, J.T. Ren, Y.Q. Miao, J.H. Wang, E.K. Wang, Biosens. Bioelectron. **26**, 3494 (2011)
39. L. Lv, Y.D. Jin, X.J. Kang, Y.Y. Zhao, Food Chem. **249**, 45 (2017)