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Building a Fluorescent Aptasensor Based on Exonuclease-Assisted Target Recycling Strategy for One-Step Detection of T-2 Toxin

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Abstract

In this work, a rapid and accurate assay was successfully developed for T-2 toxin detection based on exonuclease-catalyzed target recycling strategy. Upconversion nanoparticles (UCNPs) were conjugated with T-2 aptamer and used as signal probes, while magnetic nanoparticles (MNPs) were conjugated with the complementary DNA of T-2 aptamer (cDNA) and used as capture probes. The results reveled that good linear correlation ($R^2 = 0.9988$) was achieved for T-2 toxin detection over the concentration range of 0.1–100 ng/mL with a detection limit as low as 0.035 ng/mL ($S/N = 3$). In addition, the reliability of the proposed method was also applied to the determination of T-2 toxin contents in real food samples and the average recoveries ranged from 95.97 to 104.00%. The sensing platform developed in our study demonstrated great potential for simple and sensitive detection of T-2 toxin contents in food samples.

Keywords Fluorescence · Aptasensor · T-2 toxin · Target recycling strategy · Food safety

Introduction

T-2 toxin, a heat-stable trichothecene produced particularly by the *Fusarium species*, is widely occurred in maize, wheat, oat, beer, etc. Creppy 2002 (Chen et al. 2017; Creppy 2002). It has been proved that the consumption of T-2 toxin–contaminated food and feed could cause various pathologies on humans and animals, including lesions in hematopoietic, lymphoid, and gastrointestinal tissues and suppress reproductive organ functions (Stanford et al. 1975; Williams 1989; Yuan et al. 2014). In recent years, with the improvement of living condition, more and more people pay much attention to the safety of food products. Tremendous amount of attention has been attached to the study of T-2 toxin and it has been regarded as one of the most dangerous contaminants by the European Food Safety Authority (EFSA) (Sun et al. 2014). Considering the adverse effects and the universal existence of this hazardous toxin, there is a need to develop effective approaches for T-2 toxin detection and removal.

For the determination of T-2 toxin in foods or feedstuffs, several analytical methods such as high-performance liquid chromatography-tandem mass spectrometry (Sun et al. 2014), fluorescence assay (Khan et al. 2018), fluorescence polarization immunoassay (FPI) (Porricelli et al. 2016), ultra-performance liquid chromatography (UPLC) (Pascale et al. 2012), high-performance liquid chromatographic–mass spectrometry (LC-MS) (Flores-Flores and González-Peñas 2015), immunomagnetic bead–based enzyme-linked immunosorbent assay (IMB-ELISA) (Deng et al. 2017), ultra-performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS) (Soleimany et al. 2012), gas chromatography-tandem mass spectrometry (Kong et al. 2012), multi-immunochromatographic strip assay (ICA) (Kong et al. 2016), competitive enzyme-linked immunosorbent assay (Li et al. 2014), and ultra-performance liquid chromatography-tandem mass spectrometry (Soleimany et al. 2012) have been used. Chromatographic methods are usually highly selective and very accurate. However, these methods are relatively sophisticated, expensive, and tedious, which cannot meet the high-throughput detection required by the government and the food industry. In contrast, the immunoassay-based method

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offers rapid means of detection, but it is strongly dependent on the use of susceptible and expensive antibodies. Thus, novel, simple, low-cost, and accurate analytical methods to detect trace amounts of T-2 toxin in food/feed products are highly demanded.

Aptamers are synthetic DNA or RNA single-chain oligo nucleotides, which can bind to the targets (Seleci et al. 2016; Xuhan et al. 2018). In addition to high affinity and specificity, aptamer has more advantages compared with antibody, embracing production automation, good stability, desirable biocompatibility, lack of immunogenicity, and flexible chemical-modification (Sharma et al. 2016). Due to these appealing features, they have become a powerful tool to specifically identify various targets, such as proteins, peptides, amino acids, antibiotics, small chemicals, viruses, whole or part of cells, and even metal ions (Seok Kim et al. 2016; Taghdisi et al. 2016; Weerathunge et al. 2014). Recently, T-2 toxin aptamer was selected and identified by Chen et al. (2014) and no one has quantified T-2 levels in food based on constructing aptasensor for T-2.

Lanthanide-doped upconversion nanoparticles (UCNPs) have attracted much attention for applications in food safety analysis due to their inherent advantages (Vilela et al. 2016). Typically, UCNPs are able to absorb in the near-infrared (NIR) region and emit in the visible region of the electromagnetic spectrum. In addition, UCNPs have great chemical stability, low toxicity, high photostability, large Stokes shifts nonblinking and nonbleaching emission, sharp full width at half maxima, tunable fluorescence wavelength, and absence of autofluorescence under low-energy near-infrared (NIR) light excitation (Su et al. 2016). Up to now, UCNPs as a promising fluorophore has been successfully applied in the detection of various harmful chemicals and bacteria (Foubert et al. 2016; Kurt et al. 2016; Nguyen et al. 2016). To date, no research has been conducted to determine T-2 toxin contents in food/feed products based on UCNPs.

Herein, we developed a rapid and reliable UCNPs and magnetic nanoparticles (MNPs)-based aptasensor for T-2 toxin detection. The method established in this work would be useful for accurate and highly sensitive detection of T-2 toxin in food/feed industry.

Materials and Methods

Chemical Reagents

T-2 toxin was obtained from Sigma-Aldrich (Shanghai, China). Aflatoxin B1 (AFB1), ochratoxin A (OTA), zearalenone (ZEN), fumonisin B1 (FB1), glutaraldehyde, tetraethyl orthosilicate (TEOS), Ytterbium(III) chloride hexahydrate ($Ybcl_3{\cdot}6H_2O$), Yttrium chloride hexahydrate ($Ycl_3{\cdot}6H_2O$), and Erbium chloride hexahydrate ($Ercl_3{\cdot}6H_2O$) were purchased from Aladdin Industrial Inc. (Shanghai, China). (3-Aminopropyl) triethoxysilane (APTES), ammonium fluoride (NH_4F), 1,6-hexanediamine, and 1-octadecene (ODE) were got from J&K Chemical Technology Co., Ltd. (Beijing, China). Oleic acid (OA), iron chloride hexahydrate, iron (II) chloride tetrahydrate, cyclohexane, ammonia, ethanol, and methanol were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). T-2 toxin aptamer and its cDNA fragments were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The aptamer was purified by HPLC and suspended in deionized water from a Milli-Q device (18.2 MΩ, Millipore, MA, USA).

The detailed sequences of the oligonucleotides are as follows:

T-2 toxin aptamer: 5′-biotin-GTATATCAAGCATC GCGTGTTTACACATGCGAGAGGTGAA-3′

cDNA: 5′-SH-CGATGCTTGATATAC-3′.

Preparation and Surface Modification of Nanoparticles

Synthesis of UCNPs

Thermal decomposition procedure was applied to synthesize hexagonal $NaYF_4$: 18% Yb, 2% Er UCNPs, as reported by Sun et al. (2016). Typically, 0.80 mmol YCl_3, 0.18 mmol $YbCl_3$, and 0.02 mmol $ErCl_3$ were dissolved in 3 mL OA and 7 mL ODE in a 50-mL three-necked flask. The mixture was heated to 160 °C for 30 min and cooled down to room temperature. Thereafter, 5 mL of methanol solution containing 1 mmol NaOH and 1 mmol NH_4F was added to the mixture and stirred for 30 min. Then, the solution was heated to 300 °C under argon 1.5 h. After cooling to room temperature, the nanocrystals were precipitated by the addition of ethanol, collected by centrifugation at 5000 rpm. The precipitate from the solution was washed with methanol and ethanol several times and finally vacuum-dried at 60 °C overnight.

Surface Modification of UCNPs (AF-UCNPs)

The surface modification was according to the method reported by Stöber et al. (1968).

Synthesis of MNPs

MNPs were prepared according to a previously reported co-precipitation method (Zhang et al. 2015). 8.5 g $FeCl_3{\cdot}6H_2O$ and 3.0 g $FeCl_2{\cdot}4H_2O$ were dissolved in 38 mL hydrochloric acid in a 500 mL three-necked flask. After that, 375 mL ammonia solution was rapid added to the mixture. After vigorous stirring for 30 min, the resulting precipitates were collected with the aid of an external magnet, and then washed thoroughly three times. Finally, the obtained MNPs were dried at 60 °C in a vacuum oven overnight and stored at 4 °C for further use.

Preparation of Single-Stranded DNA–Modified UCNPs and MNPs

T-2 toxin aptamer was conjugated with UCNPs using a classical glutaraldehyde method (Ye et al. 2004). MNPs were modified with cDNA by the method reported in our previous research (Wu et al. 2017, 2018).

Fabrication of the Novel Aptasensor for the Detection of T-2 Toxin

Firstly, 100 μL of T-2 toxin aptamer-linked UCNPs and 200 μL of cDNA-MNPs were incubated in the hybridization buffer (10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 8.0) to form cDNA-MNPs-aptamer-UCNPs complex. Next, the mixed solution was incubated at 37 °C for 1 h. Then, 30 U exonuclease I was added into the mixture. Thereafter, sample solutions containing various amounts of T-2 toxin were spiked into the mixture, which was further incubated at 37 °C for 30 min with gentle shaking before deactivating by heating at 80 °C for 20 min. Subsequently, the dissociated MNPs and undissociated aptamer-UCNPs-cDNA-MNPs complexes were separated from the mixture with a magnet. The obtained precipitate was washed several times before being dispersed in PBS buffer again. Finally, the fluorescence spectra of the obtained mixture were recorded. The fluorescence signal intensities of the re-suspending solution in the absence (I_0) and in the presence (I) of T-2 were all recorded and the differences between I_0 and I (ΔI) were calculated for further use. In this study, 543 nm was selected as the detection wavelength. Each measurement was performed at least three times.

Analysis of Specificity and Selectivity

To evaluate the selectivity of the as-fabricated biosensor, other biotoxins, including AFB 1, ZEN, OTA, and FB 1, at a concentration of 1.0 ng/mL were used in the assay in place of T-2 toxin. They were all tested using the same procedures mentioned above.

Sample Preparation

Beer samples were employed to assess the application potential of the developed UCNPs-based assay for detecting T-2

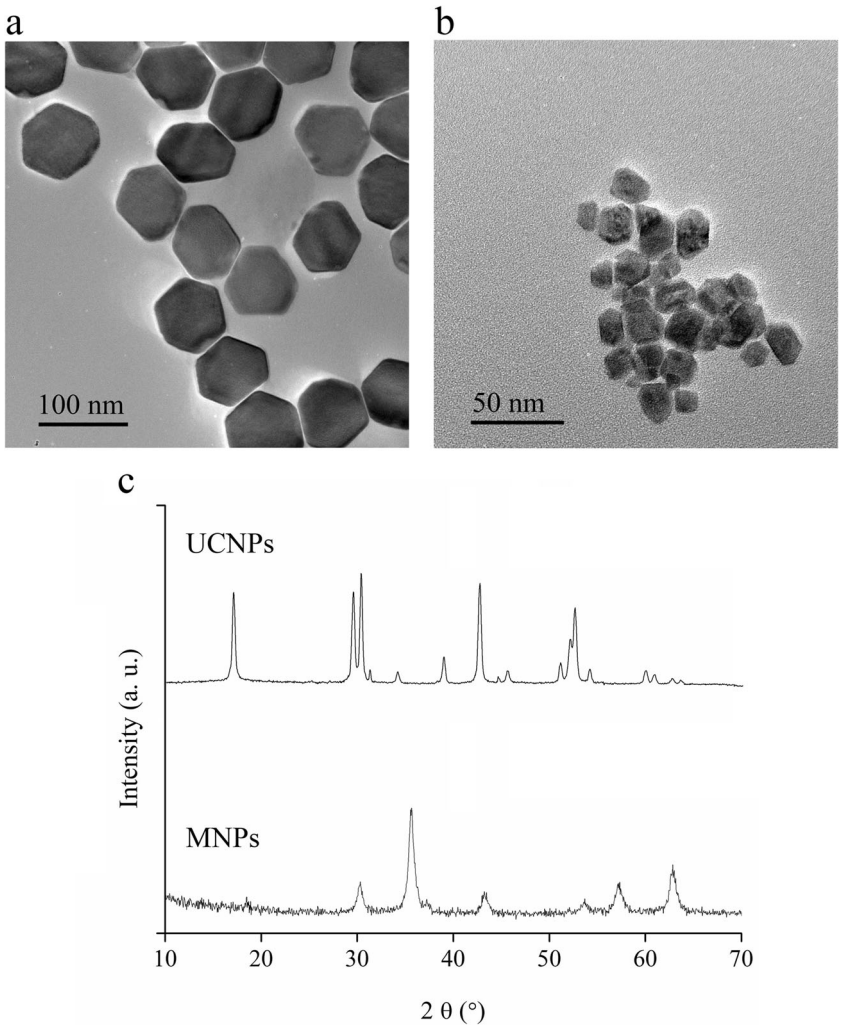

Fig. 1 TEM image of UCNPs (**a**), TEM image of MNPs (**b**), and XRD patterns of UCNPs and MNPs (**c**)

toxin in real samples. Before the detection, 50 mL of beer sample was vigorously stirred at 60 °C for 60 min for degassing purpose. After that, different concentrations of T-2 toxin were added to the prepared samples for recovery tests.

The T-2 toxin contents in the real beer samples were also validated by ELISA analysis using a commercially available T-2 toxin detection kit (Towei Chemical Reagent Co., Ltd., Shanghai, China).

Characterization of Nanoparticles

D2 PHASER X-ray diffractometer (Bruker AXS Ltd., Germany), JEM-2100 high-resolution transmission electron microscope (TEM, JEOL, Japan), and TU-1900 spectrometer (Purkinje General Corporation, Beijing, China) were used to characterize the synthesized nanoparticles in this study. Fluorescence spectra were collected with a F-7000 fluorescence spectrophotometer (Hitachi Co., Tokyo, Japan) using an external 0–1300 mW adjustable continuous wave 980 nm laser (Beijing Hi-Tech Optoelectronic Co., Beijing, China) as the excitation source.

Data Analysis

All data were expressed as mean ± standard deviation (SD) of at least three measurements throughout the study, and spectra drawings were performed using Origin 8.5 (OriginLab Inc., USA). XRD and fluorescence spectra and TEM images were collected and processed by Jade Version 5 Analysis software (Materials Data, Inc., USA), FL Solutions 2.1 software (Hitachi High-technology Co., Tokyo, Japan), and Image J2x software (NIH, USA), respectively.

Results and Discussions

Characterization

The as-prepared UCNPs and MNPs were characterized by TEM and XRD measurements. As shown in Fig. 1a and b, the morphologies of the UCNPs and MNPs are all characterized by well-dispersed with smooth surfaces. The average diameters of UCNPs and MNPs are approximately 100 nm and 20 nm, respectively. XRD measurement was performed to characterize the crystal structure of UCNPs and MNPs (Fig. 1c). The XRD pattern of UCNPs reveals that they are hexagonal, which is consistent with standard XRD results of β-phase $NaYF_4$ crystals (JCPDS Standard Card No. 16-0334) (Shikha et al. 2018). In the XRD pattern of MNPs, six characteristic peaks (2θ) at 30.20°, 35.66°, 43.14°, 53.37°, 57.03°, and 62.76°, corresponding to (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1), and (4 4 0) crystalline

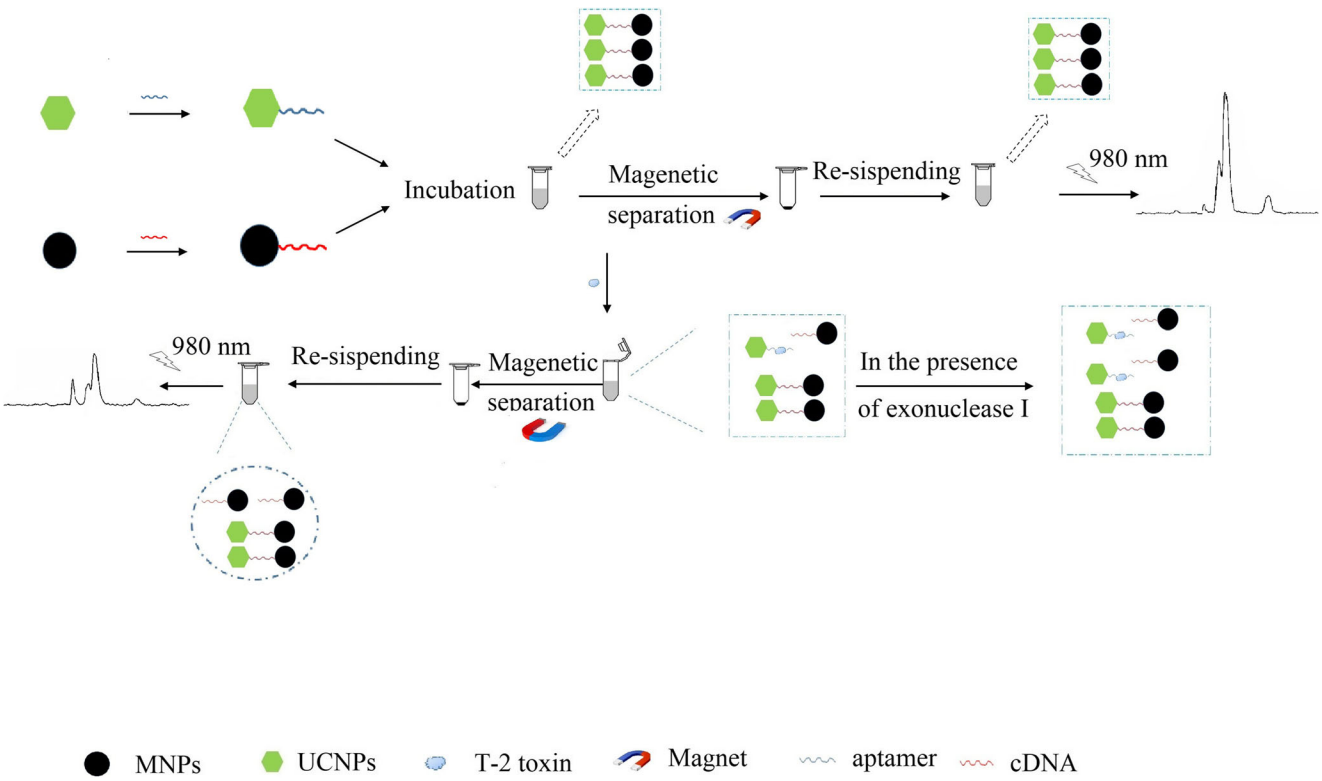

Fig. 2 Schematic diagram of the fabrication of the UCNPs-based aptasensor for sensitive detection of T-2 toxin

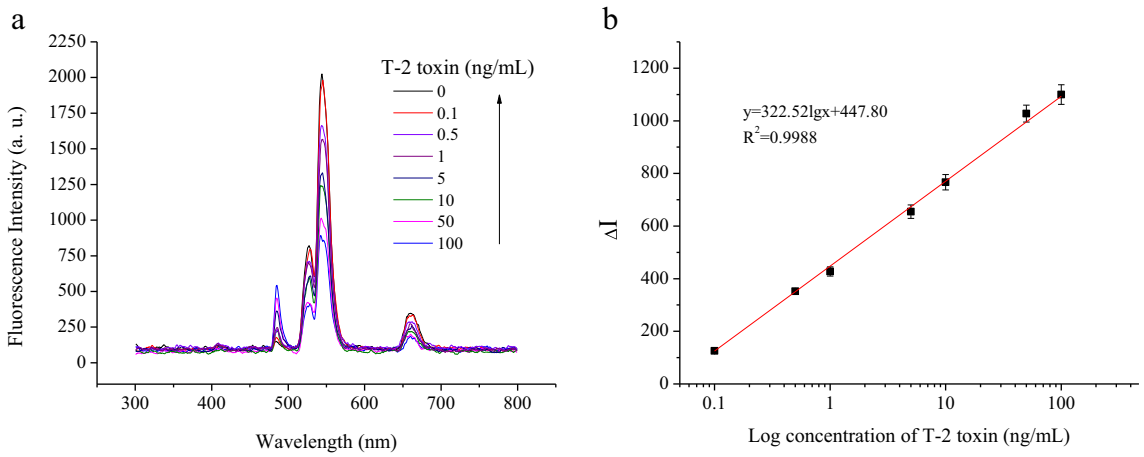

Fig. 3 Typical recording output of the developed aptasnesor exposed with different concentrations of T-2 toxin (**a**) and the linear correlation between the changed upconversion luminescent intensities and the concentrations of T-2 toxin (**b**)

planes, respectively (Wu et al. 2017), are observed, indicating that the MNPs synthesized in this work are well crystallized. The structures of the synthesized MNPs are consistent with the standard XRD diffraction pattern of Fe_3O_4 (JCPDS Standard Card No. 75-1610).

Working Principle

The working principle of the novel assay for the quantitative detection of T-2 toxin is schematically illustrated in Fig. 2. As could be seen, T-2 aptamer and its complementary DNA (cDNA) were attached on UCNPs and MNPs, respectively. By hybridizing the aptamer-UCNPs and cDNA-MNPs conjugates, stable duplex structures were formed due to the complementary base-pairing reactions. In the presence of T-2 toxin, T-2 aptamer preferentially bounded to T-2 to form a 3D stem–loop structure and dissociated from its cDNA, thereby liberating UCNPs and leading to the decrease of the fluorescence signal intensity of the re-suspending solution after magnetic separation. Exonuclease-catalyzed target recycling strategy was applied to further improve the sensitivity of the developed assay. With the addition of exonuclease I, which was specific to single-stranded DNA, T-2 toxin was liberated from the digested T-2 aptamer and conjugated to another aptamer conjugated on UCNPs. Thus, the sensitivity of the proposed assay was dramatically enhanced.

Analytical Performance

Figure 3a illustrates the typical recording output for detecting different concentrations of T-2 using the proposed assay. As can be seen, the fluorescence signal intensities gradually decrease with the increase of the T-2 concentration in the system. The standard curve, in which the decreased fluorescence signal intensity is plotted against the logarithm T-2 concentration over the range of 0.1 ng/mL to 100 ng/mL (Fig. 3b), shows a good linear relationship between changed fluorescence intensity and the T-2 concentration ($y = 322.52\lg x + 447.80$) with a correlation coefficient (R^2) of 0.9988. In this work, the half maximal effective concentration (EC50) value is calculated to be 2.07 ng/mL. The detection limit (LOD) of the sensing system is as low as 0.035 ng/mL (S/N = 3). As shown in Table 1, compared with the current existing methods, the proposed method is more sensitive.

To confirm the amplified effects of exonuclease-assisted target recycling strategy for T-2 toxin detection, a general aptasensor was fabricated under the same conditions but without exonuclease I. As shown in Fig. 4, under the same concentration of T-2 toxin, the changed fluorescence intensity of this assay was significantly bigger than that from the assay without using target recycling detection strategy. The sensitivity of the developed assay was about 5.44-fold better than that of the assay without using target recycling detection strategy.

Table 1 Comparison of the fabricated aptasensor with other methods for T-2 toxin detection

Methods	Analytical range	LOD	Recovery (%)	References
UPLC	25–100 μg/kg	8 μg/kg	91–103	Pascale et al. (2012)
LC-MS	0.1–1.0 ng/mL	0.05 ng/mL	63.5–75.8	Flores-Flores and González-Peñas (2015)
ICA	0.25–5 μg/kg	0.15 μg/kg	79.8–120	Kong et al. (2016)
UPLC–MS/MS	0.4-4000 μg/kg	2 μg/kg	95.8–97.3	Soleimany et al. (2012)
IMB-ELISA	5–75 ng/mL	2.53 ng/mL	86–99	Deng et al. (2017)
Fluorescent bioassay	0.23–17.49 μg/mL	0.19 μg/mL	93.6–106.0	Chen et al. (2014)
FPI	26–135 μg/kg	20 μg/kg	101–107	Porricelli et al. (2016)
MNPs-UCNPs assay	0.1–100 ng/mL	0.035 ng/mL	95.97–104.00	This work

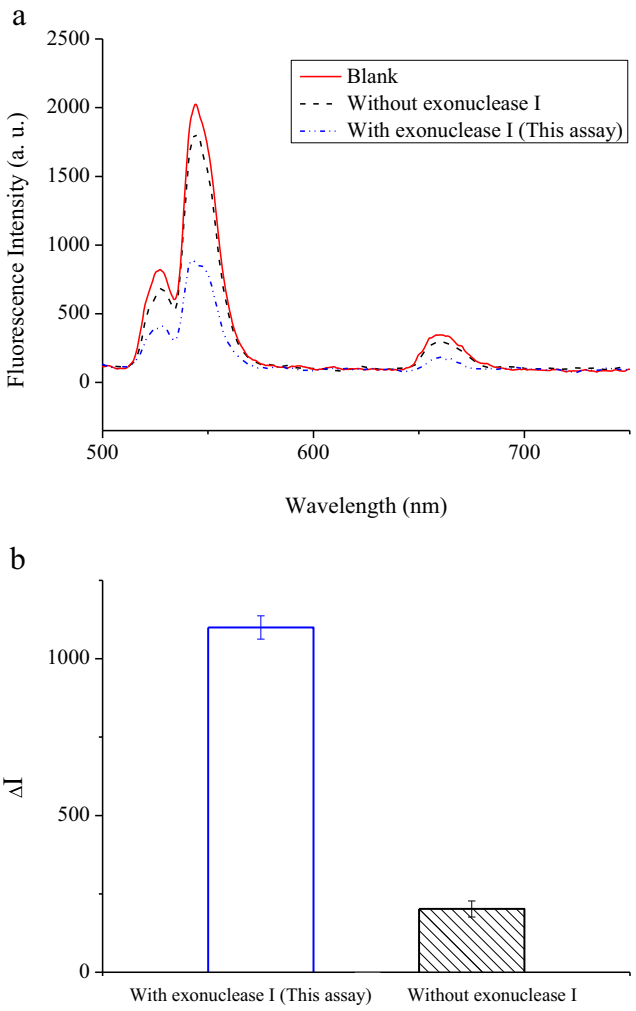

Fig. 4 Fluorescence spectra collected in the absence and in the presence of exonuclease I (this assay) when the concentration of T-2 toxin was 100 ng/mL (**a**) and comparison of the changed upconversion luminescent intensities (ΔI) recorded in the absence and in the presence of exonuclease I (this assay) (**b**)

Table 2 The determination of T-2 toxin in beer samples by ELISA and the proposed method

Background content (ng/mL)	Spiked levels (ng/mL)	Detected content (ng/mL) (mean ± SD)		
		ELISA	This method	Recovery ratio (%)
2.21	1	3.16 ± 0.25	3.24 ± 0.17	100.93
2.21	10	12.42 ± 0.73	12.36 ± 0.89	101.23
2.21	50	50.02 ± 2.67	50.11 ± 3.63	95.97
17.49	1	19.07 ± 1.16	19.23 ± 1.28	104.00
17.49	10	27.73 ± 1.75	27.59 ± 2.01	100.36
17.49	50	66.90 ± 5.82	66.78 ± 4.41	98.95
38.63	1	40.28 ± 2.51	40.41 ± 3.02	101.97
38.63	10	47.47 ± 3.38	47.55 ± 3.12	97.78
38.63	50	98.78 ± 9.75	99.15 ± 10.87	100.53

Evaluation of Specificity

To evaluate the specificity of the proposed assay for target analyte, the selectivity experiment was conducted by detecting fluorescence signal intensities when T-2 and other biotoxins (i.e., AFB 1, ZEN, OTA, and FB 1) were introduced to the constructed system. As shown in Fig. 5, obvious response (fluorescence intensity change) is obtained in the presence of T-2, while much less responses are observed in the presence of the other targets, although their concentrations (1.0 ng/mL) are ten times higher than T-2 (0.1 ng/mL). These results demonstrate the excellent selectivity of the proposed assay to T-2 toxin.

Real Sample Analysis

The feasibility of the proposed bioassay for detecting T-2 contents in beer samples was evaluated by determining the recoveries of T-2 with a standard addition method. The results of

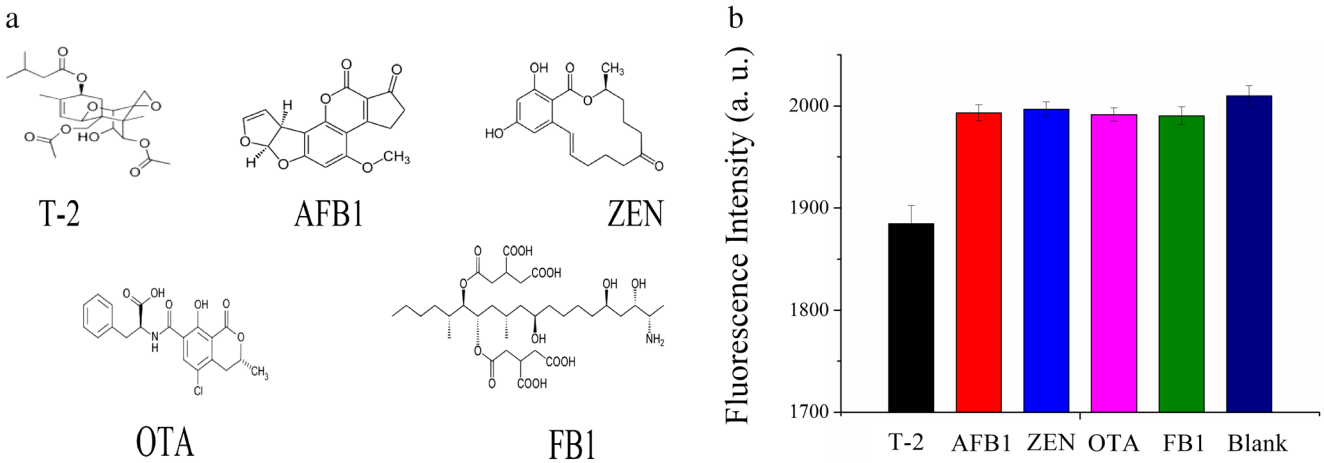

Fig. 5 Chemical structures of T-2 toxin and other toxins (**a**) and specificity of the proposed fluorescence bioassay platform toward T-2 toxin against other toxins (**b**)

recoveries and standard deviations (SD) are summarized in Table 2. As can be seen, the calculated T-2 concentrations in beer samples are almost identical to the real concentrations. All of the four spiking levels applied show good recoveries, ranging from 95.97 to 104.00%. In addition, food samples spiked with T-2 at three levels were analyzed by commercial ELISA kits to verify the practicability of the developed assay. No significant difference ($p < 0.05$) between the results obtained by the applied method and that obtained from ELISA method is observed indicating that the aptasensor developed here is reliable for the detection of T-2 in food system.

Conclusions

Summarily, we successfully developed a sensitive UCNPs-based aptasensor for fluorescence sensing of T-2 toxin. The novel strategy benefited from the unique photo-physical properties of UCNPs and high specificity of T-2 aptamer. This aptasensor demonstrated broad linear scope, ultralow detection limit, and excellent specificity for T-2. In addition, this method was successfully applied to the determination of T-2 in real food samples. These satisfactory results demonstrated that the novel fluorescent method developed on this work had great potential in sensitive and economic detection of T-2 in food samples. This novel assay could be prolonged for analysis of other poisonous ingredients in food with a substitution of specific aptamers.

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Compliance with Ethical Standards

Conflict of Interest Deyun He declares that she has no conflict of interest. Zhengzong Wu declares that he has no conflict of interest. Bo Cui declares that he has no conflict of interest. Enbo Xu declares that he has no conflict of interest. Zhengyu Jin declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human or animal subjects.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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