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Development of an indirect competitive assay-based aptasensor for highly sensitive detection of tetracycline residue in honey

Sai Wang [a], Wei Yong [b], Jiahui Liu [a], Liya Zhang [a], Qilong Chen [a], Yiyang Dong [a,*]

[a] Beijing Key Laboratory of Bioprocess, College of Life Science and Technology, Beijing University of Chemical Technology, Beijing 100029, PR China
[b] Institute of Food Safety, Chinese Academy of Inspection and Quarantine, Beijing 100123, PR China

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ABSTRACT

Tetracycline (TC) is widely used for prevention and control of animal diseases for its broad spectrum antimicrobial activity and low cost, but its abuse can seriously affect human health and may result in trade loss. Thus there is an imperative need to develop high-performing analytical technique for TC detection. In this study, we developed a biosensor based on an indirect competitive enzyme-linked aptamer assay (*ic*-ELAA). A 76mer single-stranded DNA (ssDNA) aptamer, selected by Systematic Evolution of Ligands by Exponential Enrichment (SELEX), was applied for the recognition and detection of TC in honey. The limit of detection was 9.6×10^{-3} ng/mL with a linear working range from 0.01 to 100 ng/mL toward TC in honey, and a mean recovery rate of 93.23% in TC-spiked honey was obtained. This aptasensor can be applied to detect TC residue in food with high sensitivity and simplicity, and it is prospective to develop useful ELAA Kits for TC determination in food.

1. Introduction

Antibiotic residue in foods of animal origin is one of the typical food safety issues and is deemed as an important health hazard owing to abuse and increasing antimicrobial resistance (Butaye et al., 2001). Tetracycline (TC) is a member of the broad-spectrum tetracycline group of antibiotics (tetracyclines, TCs) and can reduce affinity for prokaryotic tRNA by strong binding on the 30S ribosomal subunit (Epe et al., 1987; Spahn and Prescott, 1996). It has been widely used as veterinary drug and as feed additive, thus remains in finished food products including meat, milk, honey (Cinquina et al., 2003; Furusawa, 1999; Wasch et al., 1998). For honey, TC is often used for the treatment of bacterial brood diseases in apiculture, such as American foulbrood (*Bacillus larvae*) and European foulbrood (*Streptococcus pluton*) (Martel et al., 2006). However, there is no harmonious regulatory standard of residual TC in honey across the world. Some countries have set maximum residue limit (MRL) for TC in honey, while others have not because TCs are illegal for use with bees at any level and they do not tolerate any residue level (Li et al., 2008). For instance, the MRL for TC has been set at 300 μg/kg in Korea (Jeon and Rhee Paeng, 2008). A laboratory proposed 20 μg/kg was accepted as the recommended concentration limit but not official MRL for the screening of TC in honey in European Union (Discussion Paper Residue Expert Meeting, 2007). In China, the MRL for TC in honey was formerly set at 0.05 mg/kg (GB 14963-2003) and replaced by new regulation now (GB 14963-2011). Moreover, China is the leading producer and exporter of honey in the world. TC residue is a problem that should not be negligible as it is not only a threat to public health but also a hurdle to the international trade of apiarian products. Thus it is of great significance to develop efficient determination methods for detection of TC in honey.

In recent years, the determination of TC has been widely studied using officially accredited chromatographic methods, including high-performance liquid chromatography (HPLC) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Boscher et al., 2010; Khong et al., 2005; Lv et al., 2012; Martel et al., 2006; Nakazawa et al., 1999; Oka et al., 1994; Pagliuca et al., 2002; Samanidou et al., 2005; Viñas et al., 2004; Wang et al., 2003). They can provide simultaneous and precise results of detection, but require expensive equipments, tedious sample extraction procedures and professional technical skills. Complementary antibody-based methods for rapid detection of TC in food samples have been reported, such as enzyme-linked immuno-sorbent assay (ELISA) or gold immune-chromatographic assay (GICA) with simplicity and high-throughput screen ability. However, it left much to be desired in terms of antibody production, preservation, ethical problems with the use of animals, and the non-specific polyclonal or unsteady

* Corresponding author. Tel.: 86 10 64446260; fax: 86 10 64445535.
E-mail address: yydong@mail.buct.edu.cn (Y. Dong).

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monoclonal binding properties for *in situ* or real-time analysis (Dong et al., 2013).

Novel aptamer-based biosensor (aptasensor) has been emerged as a powerful tool which can meet the requirements of simplicity, specificity, and sensitivity for the detection of diverse substances at trace levels (Park and Paeng, 2011). Aptamers are short single-stranded oligonucleotides of DNA or RNA showing high affinity binding and high-specificity target recognition. Aptamers are also named "chemical antibody" (Song et al., 2008) due to their artificial process using Systematic Evolution of Ligands by Exponential Enrichment (SELEX) (Ellington and Szostak, 1990; Tuerk and Gold, 1990). Aptamers appear to be superior alternatives to antibodies or other biological recognition elements because they have the following advantages: (a) they can be selected *in vitro* in a stable way for various type of targets with uniform activity and not involved with immunogenicity (Jayasena, 1999; Kim et al., 2014; Nimjee et al., 2005; Tombelli et al., 2007); (b) they fold and bind upon the target molecules (Hermann and Patel, 2000; Song et al., 2008) and can be structurally modulated (Dong et al., 2013) or modified at 3′ or 5′ end (Huang et al., 2010; Pultar et al., 2009); (c) they demonstrate advantages respect to other "non-natural" receptor, such as oligopeptides, which cannot be amplified during their selection procedure (Tombelli et al., 2005). Studies on aptasensors utilizing different signal transducers, such as colorimetric (Liu and Lu, 2006; Medley et al., 2008; Stojanovic and Landry, 2002), optical (Lee and Walt, 2000; Li and Ho, 2008; McCauley et al., 2003), mass-dependant (Lee et al., 2008; Liss et al., 2002; Medley et al., 2008; Minunni et al., 2004), and electrochemical (Kim et al., 2008; Willner and Zayats, 2007) have been reported in the past few years.

Both DNA and RNA aptamer have been reported to bind specifically with TC (Berens et al., 2001; Müller et al., 2006; Niazi et al., 2008). Several studies have been reported to detect TC in food using aptamers, most of which are electrochemical aptamer-based biosensors to detect TC residue in milk (Kim et al., 2010; Zhang et al., 2010; Zhou et al., 2012), and few studies (Jeong and Rhee Paeng, 2012) are performed on microtiter plate platform which can achieve high throughput detection.

In this study, we developed an indirect competitive enzyme-linked aptamer assay (*ic*-ELAA) based on a 76mer-ssDNA (Kd = 63.6 nM) for the determination of TC in honey, and to the authors' knowledge, this study is the first report that uses validated ELAA for the determination of TC in honey. Furthermore, a biotin–streptavidin mediated system was introduced to improve target detect ability. The assay offers excellent sensitivity (the limit of detection, LOD = 9.6×10^{-3} ng/mL) with a wide linear range (0.01–100 ng/mL) and does not require complicated sample extraction steps.

2. Materials and methods

2.1. Reagents

A single-strand 76mer DNA aptamer was custom synthesized with 3′-end biotinylated modification by Sangon Biotech Co., Ltd. (Shanghai, China), and has the following sequence (Fig. 1): 5′-CGTACGGAATTCGCTAGCCCCCCGGCAGGCCACGGCTTGGG TTGGT CCCACTGCGCGTGGATCCGAGCTCCACGTG-3′-biotin (Mw. (23747.43), mp (83.77 °C)). TC–BSA was obtained from NanKai Biotech Co., Ltd. (Hangzhou, China). Tetracycline standard and Hammerstein bovine casein were purchased from Sigma-Aldrich (St. Louis, MO, USA) and 2-amino-2-(hydroxymethyl)-1, 3-propanediol (Tris) was purchased from Sigma-Aldrich (Shanghai, China). Bovine serum albumin (BSA), horseradish peroxidase labeled Streptavidin (SA–HRP) and 3,3′,5,5′-tetramethylbenzidine dihydrochloride (TMB) were purchased from

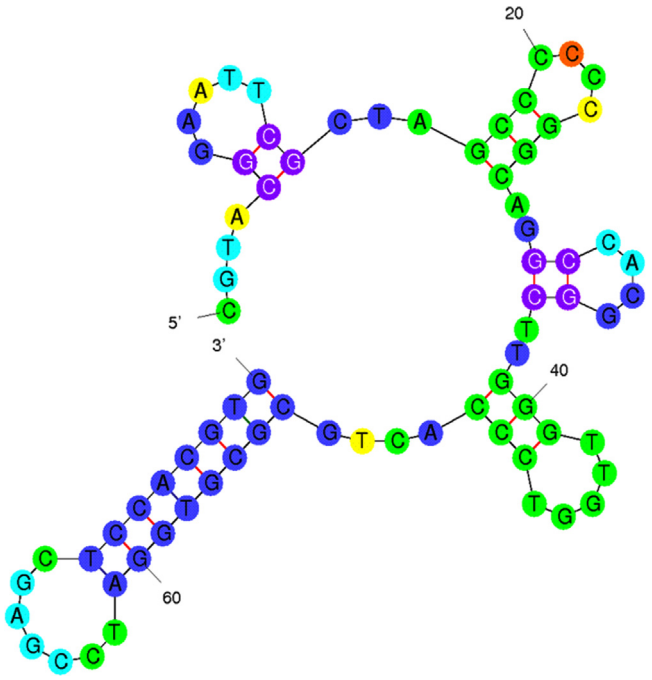

Fig. 1. Secondary structure of the 76mer ssDNA aptamer. The structure was predicted by m-fold program according to the free minimization algorithm. Color annotation means the probability.

KPL (Gaithersburg, MD, USA). All chemicals were of analytical grade and ultrapure water was prepared by Thermo Scientific Barnstead GenPure Water Purification System (Thermo Electron LED GmbH, Stockland 3, D-56412 Niederelbert). All buffers were filtered using 0.22 μm membrane filter before use.

2.2. Instrumentation

ELAA was performed in 8 well Flat-Bottom Immuno Plate (Nunc, Denmark). Absorbance was measured using *ELx800* absorbance microplate reader (BioTek Instruments, Inc., Winooki, Vermount, USA) at both 450 nm and 630 nm. The plates were washed using *ELx50* microplate strip washer (BioTek Instruments, Inc., Winooki, Vermount, USA). The absorbance of honey at 280 nm was obtained using Nanodrop2000C (Thermo Fisher Scientific Nanodrop2000C, Wilmington, Delaware, USA). Centrifugation was performed in Sigma refrigerated centrifuge 3K15 (Sigma Laborzentrifugen GmbH, An der Unteren Söse 50, D-37520 Osterode, Germany).

2.3. Optimization of assay conditions

Prior to the competition assay, the optimization was carried out to achieve an appropriate absorbance of negative control (non-competitive assay) around 1.0 in order to match with the sensitivity of the microplate reader and get an inhibition curve with relatively larger slope. The absorbance of the negative control was compared while varying concentrations of binder (aptamer) and competitor (TC–BSA). The TC–BSA conjugate (initial molar ratio is 3:1) was prepared in a concentration of 0.5–10 μg/mL (ppm) and the aptamer was diluted to 1–20 nM. Moreover, in light of the alteration of the recognition element with respect to ELISA, it was necessary to characterize the effects of coating buffer, blocking agent, binding buffer and concentration of SA–HRP. Thereupon five types of coating buffer were compared, referring to those usually used in the ELISA. They were 50 mM bicarbonate buffer (CB, pH 9.6), 10 mM Tris–HCl buffer (Tris, pH 8.0), 10 mM phosphate buffer saline (PBS, pH 7.4), 100 mM phosphate buffer (PB, pH 7.2) and

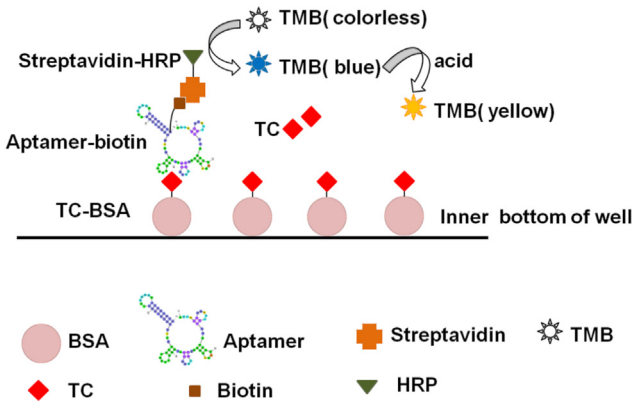

Fig. 2. Schematic illustration of the aptasensor based on indirect competitive ELAA for TC detection. Competition between immobilized TC–BSA and free TC in standard or honey solution for the biotinylated aptamer and incubation with the sterptavidin–HRP conjugate, and subsequent enzyme label detection.

100 mM citrate-phosphate buffer (CPB, pH 5.0). Two different blocking agents of BSA and Hammerstein bovine casein at various concentrations were assessed. The aptamer was diluted in three kinds of binding buffers, Buffer A (100 mM NaCl, 20 mM Tris–HCl (pH 7.6), 2 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM KCl, 0.02% Tween-20, (Niazi et al., 2008)), Buffer B (10 mM Tris–HCl, pH 7.6), Buffer C (50 mM NaCl, 10 mM Tris–HCl (pH 7.6), 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 2.5 mM KCl, 0.02% Tween-20). A thermal treatment was performed by heating the aptamer at 95 °C for 10 min and then cooling rapidly in ice for 10 min before the labeled ssDNA binding to TC. And the SA–HRP was diluted into three concentrations of 1 μg/mL, 3 μg/mL and 6 μg/mL.

2.4. Indirect competitive enzyme-linked aptamer assay(ic-ELAA)

As shown in Fig. 2, the plates were firstly coated with 100 μL/well of the TC–BSA conjugate in 10 mM Tris–HCl (pH 8.0), and incubated overnight at 4 °C. To remove unbound TC–BSA, wells were washed three times with 300 μL/well of wash buffer Tris–HCl-T (10 mM Tris–HCl, pH 7.6, 0.05% Tween-20). The plates were then blocked with 200 μL/well of 0.25% (w/v) Hammerstein bovine casein in 10 mM Tris–HCl (pH 8.0) for 30 min at 37 °C and the unbound protein was removed. Subsequently, 50 μL/well biotinylated aptamer in Buffer A and 50 μL/well various concentrations of TC solution from 5×10^{-4} to 1×10^4 ng/mL were added and the binding was allowed for 75 min with mild shaking at room temperature (RT). After washing, 100 μL/well SA–HRP (6 μg/mL) in 10 mM Tris–HCl (pH 8.0) was added to the plates, and the incubation was accomplished after 45 min at 37 °C. Finally, 100 μL/well TMB solution was added and incubated for 20 min at 37 °C until the color of solution changed from colorless to blue. Then the color reaction was stopped by adding 50 μL of 2 M H_2SO_4 and the color turned to yellow. The absorbance was measured immediately at 450 nm with 630 nm as the reference wavelength to reduce the interference of measurement and electro-circuit (Lu, 2011; Matsuda et al., 2006). Each step was protected from light. The final absorbance of the competition system, denoted by B, was consistent with the result of absorbance at 450 nm minus absorbance at 630 nm. B_0 means the absorbance value of the negative control. The absorbance values were converted into their corresponding test inhibition values (B/B_0).

2.5. Detection of TC in honey by using indirect competitive ELAA

Honey samples were purchased from local supermarket and stored at RT before use. To remove the metal ions in honey such as calcium, iron, magnesium and zinc (Huq et al., 2006), honey samples (5 g) were mixed with 20 mL of 0.1 M McIlvaine-Na_2EDTA buffer (M-E, 12.93 g of citric acid, 37.33 g of Na_2EDTA, 22.38 g $Na_2HPO_4 \cdot 12H_2O$ in 1 l of ultrapure water, pH 4.0) for 5 min using a vortex mixer and then centrifugated at 4 °C for 20 min at 4000 rpm. Then 4 M NaOH was added dropwise to the supernatant to adjust the pH to 7.6. Finally, samples were stored at 4 °C in the dark and diluted with 10 mM Tris–HCl (pH 7.6) (sample:Tris–HCl = 1:1) before use. McIlvaine-Na_2EDTA buffer is a common buffer for honey sample treatment reported using ELISA. However, absorption peak at 280 nm was found by Nano-drop2000C (data not shown). Thus, additional 20% trichloroacetic acid (TCA) was added as acidic deproteination agent. And two methods for pretreatment of samples were compared concerning the presence or absence of TCA. TC standard was dissolved and diluted in concentrations from 5×10^{-4} to 1×10^4 ng/mL using the honey solution. The resulting matrix-matched standard TC in honey solutions were used in this study.

3. Results and discussions

3.1. Optimization of assay conditions

TC is amphoteric compound as it has both Lewis base and Lewis acid functional groups (Novák-Pékli et al., 1996). It has a high affinity to form chelate compounds with metal ions (Baek and Paeng, 2008; Jin et al., 2007; Wessels et al., 1998), and proteins including the frequently-used blocking agent BSA (Bi et al., 2005; Keswani et al., 2013; Khan et al., 2002), which consequently affects the combination between TC and aptamer in competition and has subsequent consequences to the sensitivity of determination. Based on the above, detailed works were conducted focusing on optimizing working conditions that affect the absorbance of negative control and sensitivity of the assay. In addition, significance analysis was made between different conditions and added to Fig. 3. Immobilized TC–BSA used at different concentrations (0.5–10 μg/mL) was exposed to various concentrations (1–20 nM) of biotinylated aptamer. The satisfactory coating concentration of wells was achieved using 1, 2, and 5 μg/mL of the TC–BSA conjugate and 10 nM aptamer. Furthermore, other conditions were optimized utilizing the optimized concentration of binder and competitor. As is shown in Fig. 3(A), 10 mM Tris–HCl (pH 8.0) showed better performance as a coating buffer than others on account of the absence of metal ions and the high pH value promoting TC–BSA to be absorbed onto the bottom of the wells. Binding reactions were conducted using the optimized concentrations of TC–BSA and aptamer in different binding buffers, and Buffer A showed the best performance (Fig. 3(B)). Buffers B and C were applied, with the purpose of reducing the metal ions. However, as shown in the result, it is necessary to reserve a certain degree of metal ions for aptamer, because they can contribute to the stability and structure of DNA (Marathias and Bolton, 1999; Owczarzy et al., 2008; Seo et al., 2012). In addition, Buffer A was used to dilute aptamer in advance, then 50 μL/well aptamer in Buffer A and 50 μL/well TC in 10 mM Tris–HCl were mixed in the wells, thus the metal ions have little effect on TC. And pH 7.6 was chosen to protect the ssDNA from degradation as higher or lower pH values may negatively affect hydrogen bond stability. The feasible concentration of SA–HRP was found to be 6 μg/mL (Fig. 3(D)), slightly higher than 5 μg/mL as recommended in KPL's product sheet. In addition, two different blocking agents at various concentrations were evaluated and 0.25% (w/v) Hammerstein casein solution prepared in 10 mM Tris–HCl (pH 8.0) resulted in a desired absorbance of negative control (Fig. 3(C)). BSA is a common blocking agent in immunoassays to block non-

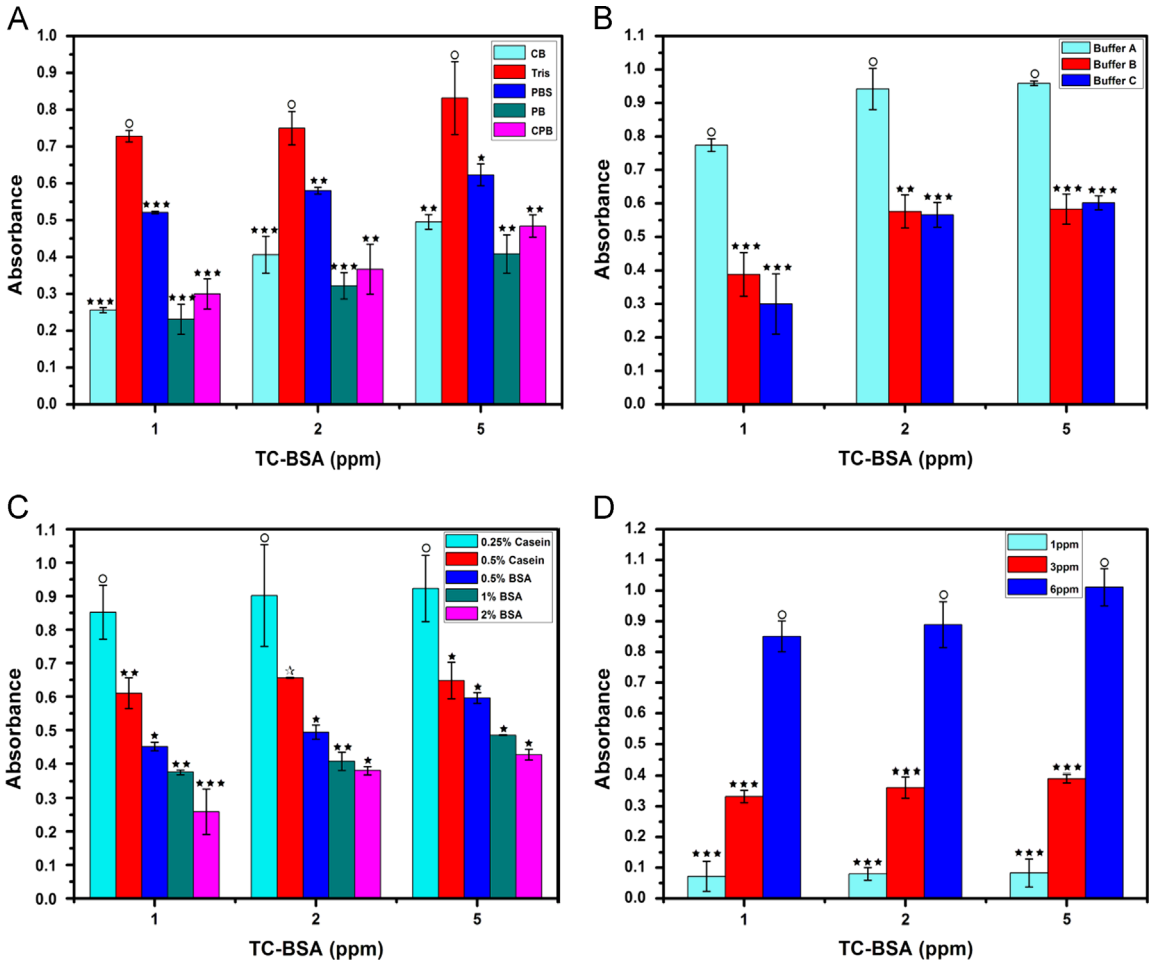

Fig. 3. Optimization of conditions. Detailed working conditions were evaluated with 1, 2, and 5 ppm of TC–BSA and 10 nM aptamer. (A) Absorbance of 1, 2, and 5 ppm TC–BSA in different coating buffers. They were bicarbonate buffer (CB), Tris–HCl buffer (Tris), phosphate buffer saline (PBS), phosphate buffer (PB) and citrate-phosphate buffer (CPB). (B) Absorbance of 1, 2, and 5 ppm TC–BSA with 10 nM aptamer in three kinds of binding buffers. (C) Absorbance of 1, 2, and 5 ppm TC–BSA under the condition of different blocking agents. (D) Absorbance of 1, 2, and 5 ppm TC–BSA utilizing various concentrations of SA–HRP. Significance analysis was characterized by *P* value using two-tailed *T*-tests. The symbol ° denotes control group, ☆ denotes $P > 0.05$, ★ denotes $P < 0.05$, ★★ denotes $P < 0.01$, and ★★★ denotes $P < 0.001$.

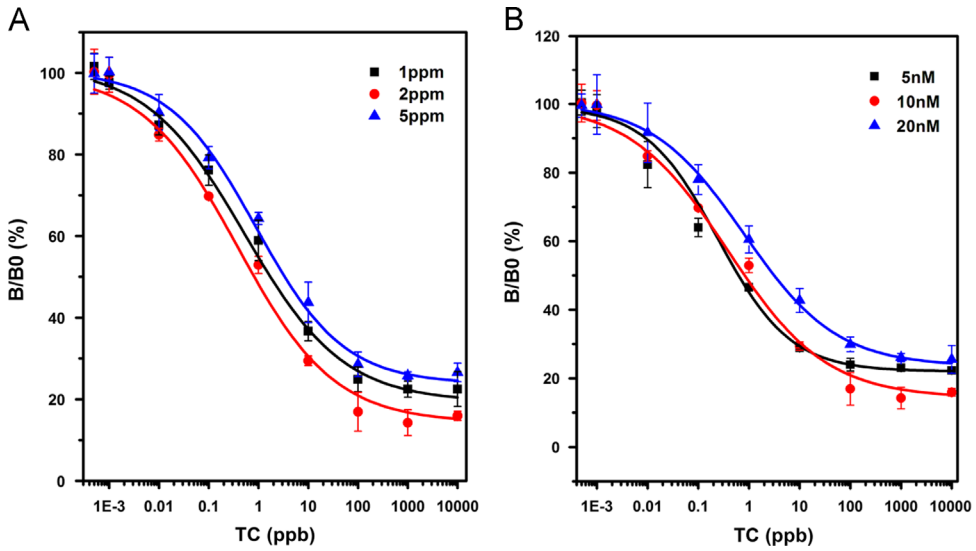

Fig. 4. Indirect competitive ELAA in buffer. (A) Inhibition curves for TC by *ic*-ELAA using various concentrations of the TC–BSA conjugate (1, 2, and 5 ppm) with 10 nM aptamer. (B) Inhibition curves for TC by *ic*-ELAA using various concentrations of aptamer (5, 10, and 20 nM) with 2 ppm of TC–BSA. Data points are the average plus ± one standard deviation ($n = 3$).

specific binding, however it is reported that small molecules or drugs, such as tetracycline, have the ability to interact with serum albumin including BSA and HSA and this character is preferred to be used in drug delivery research (Bi et al., 2005; Keswani et al., 2013; Khan et al., 2002). Moreover, 10 mM Tris–HCl was also used to dilute the blocking agent in this assay, and the absence of metal

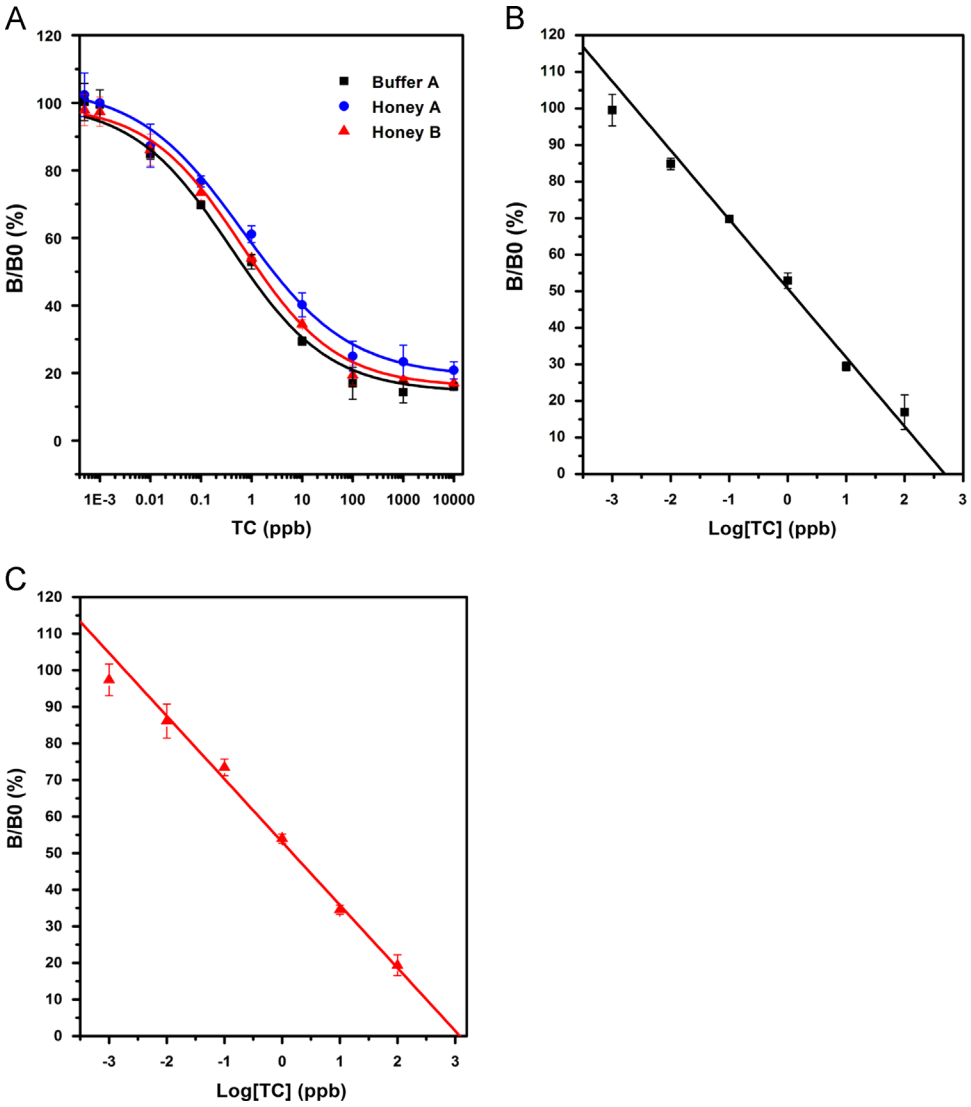

Fig. 5. Calibration curves for TC. (A) Inhibition curves using 2 μg/mL of TC–BSA and 10 nM aptamer in Buffer A and honey. Honey A represents the pretreatment of honey with McIlvaine-Na_2EDTA buffer and Honey B stands for the pretreatment of honey with both McIlvaine-Na_2EDTA buffer and trichloroacetic acid. (B) Calibration curve for TC in Buffer A. (C) Calibration curve for TC in honey B. Data points are the average plus ± one standard deviation (n=3). "ppb" means ng/mL.

ions in the dilution buffer contributed to facilitate the binding between TC and serum album because metal ions and TC compete to bind with BSA (Bi et al., 2005). Thus the results in this study can be explained that TC interacted with the blocking agent BSA, consequently decreasing the absorbance.

3.2. Detection of TC in buffer by indirect competitive ELAA

After optimization, the indirect competitive assay was performed. The assay sensitivity is dependent on the binder and competitor, hence, three coating concentrations of the TC–BSA conjugate (1, 2, and 5 μg/mL) were compared and three concentrations of aptamer (5, 10, and 20 nM) were used to investigate the sensitivity for TC detection. The 2 μg/mL concentration of TC–BSA and 10 nM concentration of aptamer were found to provide optimum sensitivity and sufficient absorbance values for an easily detectable signal. As is shown in Fig. 4(A), the 2 μg/mL of TC–BSA presented a curve with a steeper slope, while 1 μg/mL and 5 μg/mL of TC–BSA brought out a slowly changing curve. According to the theoretical analysis, the lower coating concentration (1 μg/mL) results in more non-specific adsorption, while the higher coating concentration (5 μg/mL) gives rise to low sensitivity in competition. The results in Fig. 4(B) demonstrated that sensitivity increased as lower concentrations of aptamer were used. However, 5 nM concentration of aptamer showed a relatively narrow linear range than 10 nM because the lower concentration of aptamer was more likely to be mostly occupied with the increase of TC in the competition assay. A thermal treatment of ssDNA at 95 °C for 10 min and then cooling rapidly in ice for 10 min (fast cool anneal) was performed in order to block the aptamer in its unfolded structure (Jeong and Rhee Paeng, 2012) because single DNA or RNA tends to be more ordered and comes into adaptive fold from uncertain conformation to energy-efficient three-dimension structures when meeting with their targets in the solution (Bishop et al., 2007; Hermann and Patel, 2000). Moreover, Marathias found that for several short DNAs in their study, fast cool anneal processed a single DNA quadruplex conformation while slower cool anneal (cool down to room temperature slowly after thermal denaturation) produced mixtures (Marathias and Bolton, 1999). Based on the above, a fast cool anneal was adopted to promote the structure of aptamer ordering and save analytical time.

Table 1
Recovery study.

Spiked concentration (ng/mL)	Measured concentration (ng/mL, mean ± SD)	Recovery (%, mean)	RSD (%)	Mean recovery (%)
0.1	0.085 ± 0.004	85.38	4.42	
1	0.862 ± 0.023	86.23	2.66	93.23
10	10.807 ± 0.22	108.07	2.01	

SD: standard deviation (n=3).
RSD: relative standard deviation.

3.3. Detection of TC in honey by using indirect competitive ELAA

To remove the metal ions in honey, Na_2EDTA was used as it has a greater affinity for cations than TC (Anderson et al., 2005) and TCA was well known as a deproteination agent. TC is decomposed to anhydro-form under strong acidic condition (below pH 2), so McIlvaine-Na_2EDTA buffer was adjusted to pH 4.0 (Oka et al., 2000). In addition, it is reported that macrolides are not stable in acidic conditions (Wang, 2004), and the acidic pH value is conducive to reducing the cross-activity in ELAA.

The dose–response curves were also explored using 2 μg/mL of TC–BSA and 10 nM of aptamer. In this study, two kinds of sample treatments were compared. The competition in honey with the pretreatment using both McIlvaine-Na_2EDTA and TCA showed preferable competition curve (Fig. 5(A)). The dose–response curves in buffer and honey with better processing method were calculated as following: (1) B/B_0 (%) of TC in buffer = $-18.88 \times \log[TC] + 50.75$, R^2=0.9867 (Fig. 5(B)); (2) B/B_0 (%) of TC in honey = $-17.22 \times \log[TC] + 53.05$, R^2=0.9652 (Fig. 5(C)). The LOD of 7.8×10^{-3} ng/mL and a linear range of 0.01–100 ng/mL were achieved in buffer. Additionally, the LOD and the linear range were 9.6×10^{-3} ng/mL and 0.01–100 ng/mL in honey. The LOD was defined as the concentration corresponding to 3 standard deviations below the mean absorbance from negative control. The results indicate that the *ic*-ELAA method in this assay yields similar LOD values in buffer and honey without obvious matrix effect.

3.4. Recovery and cross-reactivity study

The accuracy of the assay detecting TC in honey samples was evaluated by recovery study. Free TC (0.1 ng/mL, 1 ng/mL and 10 ng/mL) were spiked in honey solution and mean percentage recoveries were analyzed to be 85.38–108.07% with an average value of 93.23% in honey with simple sample preparation (Table 1).

The aptamer used in this assay was assessed for cross-reactivity against a range of TCs (oxytetracycline, tetracycline, doxycycline) when it is selected by SELEX (Niazi et al., 2008). The results of specificity tests showed the aptamer we used in our assay yield a measurable specific binding to TC than its structural similarities, and the ssDNA bound to TC was more than twice as the others (data not shown).

3.5. Stability of tetracycline in honey

Efforts were made to keep TC stable in honey for subsequent detection. Honey was kept in the dark, since TC is light-sensitive. In addition, some research data have confirmed the stability of TC in honey. Münstedt applied HPLC to analyze the mixture of oxytetracycline, chlortetracycline, and tetracycline, and more than half of the original concentration of chlortetracycline and tetracycline were observed even after 10 months of storage at ambient temperatures (Münstedt et al., 2002). Martel observed no loss of TC content by storing honey with the analyte for 2 months at 4 °C (Martel et al., 2005).

Most existing detection using HPLC can obtain LODs of 0.1–20 ng/g with linear ranges from tens to near one thousand ng/mL (Hakuta et al., 2009; Li et al., 2008; Peres et al., 2010; Vidal et al., 2009), while rapid detection methods, such as ELISA and electrochemical luminescence, can provide lower LODs (below 10 ng/mL or even below 1 ng/mL) but relatively narrow linear ranges (Guo and Gai, 2011; Jeon and Rhee Paeng, 2008; Pang et al., 2005; Reybroeck et al., 2007). Moreover, to further improve detection method for TC, Jeong developed a direct competitive ELAA using two different aptamers to detect TC in milk (Jeong and Rhee Paeng, 2012). However, the developed method was not superior to ELISA in terms of specificity, LOD and linearity range.

Compared with other reported analytical data for detection of TC in honey, our method demonstrated remarkable advantages in terms of detection limit and linear range.

4. Conclusions

In conclusion, an indirect competitive assay-based aptasensor for detection of tetracycline in honey was developed. In this study, we utilized buffers with metal ion concentration as low as possible to ensure the combination between TC and aptamer and obtained competition curves both in buffer and honey samples. Our proposed method indicates superior sensitivity (LOD of 9.6×10^{-3} ng/mL), linear range (0.01–100 ng/mL) and high recovery rate (85.38–108.07% in TC spiked honey) compared to other methods. The proposed method is promising to provide a simple and highly sensitive screening for high throughput TC monitoring, and the scheme is envisaged to be utilized for the determination of other kinds of antibiotics in various foods.

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