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A membrane-based fluorescence-quenching immunochromatographic sensor for the rapid detection of tetrodotoxin

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ABSTRACT

A membrane-based fluorescence-quenching immunochromatographic sensor (FQ-ICS) was developed to detect tetrodotoxin (TTX) in as little as 12 min by utilizing fluorescence resonance energy transfer (FRET). Compared with traditional ICS for detecting TTX, this FQ-ICS provided a positive readout of the signal intensity as well as higher sensitivity. The signal detected during tests increased along with increasing TTX concentrations within a determinate concentration range. The limit of detection (LOD) of the method was 0.78 ng/mL, a value more than 20 times lower than the latest reported value using colloidal gold strips for TTX detection. Furthermore, the detection linear range (DLR) was 3.13–50 ng/mL. Recovery rates for the FQ-ICS used to detect TTX in spiked samples ranged from 61.3% to 70.4%. These results indicate that the FQ-ICS is highly sensitive and suitable for the rapid detection of TTX.

1. Introduction

Tetrodotoxin (TTX) is a potent, low molecular weight (319 Da) marine neurotoxin that is found in a variety of species, such as puffer fish, sea slugs, star fish, blue-ringed octopuses, crabs, frogs, gastropods, and newts in addition to bacterial sources (Bane, Lehane, Dikshit, O'Riordan, & Furey, 2014) The most relevant encounters with the toxin involve puffer fish, which is commonly eaten by people living in coastal regions. As a result, many cases of poisoning are reported each year (Lago, Rodríguez, Blanco, Vieites, & Cabado, 2015) due to the consumption of puffer fish meat that has been polluted by TTX which is concentrated primarily in the fish's ovaries and liver (Bane et al., 2014; Noguchi, Arakawa, & Takatani, 2006). TTX is both heat stable and water soluble, making it difficult to negate its toxic effects in cooking conditions (Ling et al., 2015; Saoudi et al., 2007). Consumption of TTX acts to block voltage-gated sodium channels in excitable tissues such as muscles and nerves (How, Chern, Huang, Wang, & Lee, 2003; Noguchi & Arakawa, 2008), resulting in nausea, vomiting, diarrhea, weakness of the limbs, nerve paralysis, respiratory failure, and even death (Chen, Hsieh, & Hwang, 2016; Narahashi, Moore & Poston, 1967). The median lethal dose (LD_{50}) of TTX in mice is 2–10 μg/kg when administered by intraperitoneal injection and 10–14 μg/kg by subcutaneous injection (Alcaraz, Whipple, Gregg, Andresen, & Grant, 1999). This indicates that only about 0.5 mg of TTX can lead to death by poisoning in an adult human. Based on these dangers, rapid and precise detection of TTX is necessary.

In recent years, detection methods for TTX have become

Abbreviations: FQ-ICS, fluorescence-quenching immunochromatographic sensor; TTX, tetrodotoxin; FRET, fluorescence resonance energy transfer; LOD, detection limit; DLR, detection linear range; LD50, median lethal dose; MBA, mouse bioassays; LC-MS, liquid chromatography-mass spectrometry; SPR, surface plasmon resonance; ECL, electrochemiluminescence sensor; ELISA, enzyme-linked immunosorbent assay; LFICS, lateral flow immunochromatographic sensors; FQ-ICS, fluorescence quenching immunochromatographic sensor; FM, Fluorescent microsphere; AuNPs, gold nanoparticles; mAb, monoclonal antibody; AuNPs-mAb, gold nanoparticles labeled mAb; FM-BSA-TTX, Fluorescent microsphere labeled BSA-TTX; FM-BSA, Fluorescent microsphere labeled BSA.

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increasingly diverse, and include mouse bioassays (MBA) (P. A. Hwang et al., 2005), liquid chromatography-fluorescence detection (Yu, Yu, Tam, & Yu, 2010), liquid chromatography-mass spectrometry (LC-MS) (Cho et al., 2012), surface plasmon resonance (SPR) (Yakes, Deeds, White, & Degrasse, 2011; Yakes, Kanyuck, & DeGrasse, 2014), electrochemiluminescence sensor (ECL) (Shang, Liu, & Wang, 2016), enzyme-linked immunosorbent assay (ELISA) (Ling et al., 2015), and so on. Each method listed has its own character, pros, and cons. The detection linear range (DLR) and the limit of detection (LOD) of several of these methods are listed in Table 1. As could be expected, LC-MS, ECL, and SPR are all highly sensitive, but their use is often restricted due to the high equipment expenses and amount of time required. ELISA has been the primary immunoassay technique used for detection of TTX because it is both sensitive and precise. However, this method also has the disadvantages of being both costly and time-consuming. In contrast, lateral flow immunochromatographic sensors (LFICS) are a disposable, low-cost, quick, specific, and sensitive method for TTX detection. As shown in Table 1, a lately developed competitive format colloidal gold strip had an LOD for TTX of 20 ng/mL (Ling et al., 2015). However, despite its advantages this method was not found to be satisfactory due to sensitivity limitations as well as the negative readout of the signal intensity. Recently, Fluorescent nanoparticles have been utilized in conjunction with LFICS and have displayed high sensitivities (Shi et al., 2015; Fu et al., 2014; Fu et al., 2013; Liu et al., 2015; Wang et al., 2014; Zhang et al., 2016; Zhou et al., 2014). Furthermore, positive signal intensity readouts have been obtained by using a novel fluorescence quenching immunoassay developed to detect small analytes (Q. Q. Fu et al., 2013). Fluorescence reader has become a useful fluorescent quantitative detection device in recent years. Therefore, fluorescence quenching immunoassay has a more broad application prospect. In the present work we aimed to develop an AuNPs-based fluorescence quenching immunochromatographic sensor (FQ-ICS) that provides a rapid, economic, sensitive, positive readout, and quantitative (or semi-quantitative) tool for the detection of TTX to improve food safety.

2. Materials and methods

2.1. Materials and reagents

TTX (99.99% purity by HPLC), Anti-TTX-monoclonal antibodies (TTX-mAb, antibody titer determinated by i-ELISA reaching 1: 2.56×10^5) and BSA-TTX (2.5 mg/mL) were obtained from Shenzhen Academy of Metrology and Quality Inspection (Shenzhen, China). Puffer fish (T. fasciatus) was purchased from Lianyungang, Jiangsu Province, China. TTX ELISA test kit was purchased from Jiangsu Meizheng Bio-Tech Co., Ltd (China). Nitrocellulose (NC) membrane (HF18002S25), conjugation pad, and sample pad were purchased from Millipore (Shanghai, China). Fluorescent microsphere (FM) with an excitation wavelength of 468 nm and emission wavelength of 508 nm were purchased from Suzhou Vdo Biotech (Suzhou, China). AuNPs-mAb quencher was generated in our laboratory. Ultrapure water (resistivity: 18.2 MΩ cm) produced by a Milli-Q Ultra Pure System (Millipore, USA) was used throughout this study. All chemicals used were of analytical grade or higher.

Table 1
Comparison of major methods reported for the determination of TTX.

Method	LOD (ng/mL)	DLR (ng/mL)	Reference
LC-MS	0.32	2–1200	(Cho et al., 2012)
ECL	0.10	0.5–5000	(Shang et al., 2016)
SPR	0.09	–	(Betsy Jean Yakes et al., 2014)
ELISA	4.44	5–500	(Ling et al., 2015)
colloidal gold strip	20.0	–	(Ling et al., 2015)

2.2. Apparatus

Apparatus used during experiments included a centrifuge 5810 R (Eppendort, Germany), a Milli-Q Ultra Pure System (Millipore, USA), a magnetic stirrer (Beike, Beijing), a JEOL 2100F transmission electron microscope (TEM, Japan), a Synergy H1 Hybrid Multi-Mode Microplate Reader (Bio-Tek Instruments, Inc.), a programmable strip cutter HGS201 (AUTOKUN, China), a fluorescence reader AFS-1000 (LAB, China), a XYZ3060 platform consisted of motion control with Biostrip Dispenser HGS102 and Airjet HGS102 (BioDot, China), and a fluorescence camera invented by our lab.

2.3. Preparation of gold nanoparticles (AuNPs) and gold nanoparticles labeled mAb (AuNPs-mAb) quencher

AuNPs with a mean particle diameter of 20 nm were produced by reducing gold chloride ($HAuCl_4$) with 1% sodium citrate (Na_3Ct) (Ji et al., 2007). In summary, 50 mL of ultrapure water was heated to boiling on a hotplate with a magnetic stirrer. 1.0 mL of 1% $HAuCl_4$ was quickly added, followed by 1.8 mL of 1% Na_3Ct 1 min later, and the solution was stirred for 20 min. After that the solution was allowed to cool to room temperature and then transferred to a 50-mL volumetric flask, diluted, and mixed.

AuNPs-mAb quencher was prepared as follows: 10 μL TTX-mAb was diluted to 200 μL in ultrapure water and added into 10 mL of a colloidal gold solution. The mixture was then adjusted to pH 8.5 using 0.25 M K_2CO_3. After an incubation at room temperature for 30 min, 1 mL 10% BSA was added to the solution which was then incubated again at room temperature for 30 min. Finally the mixture was centrifuged at 9000 RCF for 15 min and the precipitate was re-suspended in 600 μL PBS (0.015 M, pH 7.4, and containing 20% (w/v) sucrose, 20% (w/v) trehalose, 1% (w/v) PVP K-40, 1% (w/v) BSA, and 1% Tween-20), and was stored at 4 °C for further use.

2.4. Preparation of fluorescent microsphere labeled BSA-TTX (FM-BSA-TTX) and fluorescent microsphere labeled BSA (FM-BSA)

FM-BSA-TTX was prepared as follows: 100 μL FM was dispersed in 400 μL of ultrapure water. Next, both 13.4 μL EDC (1 mg/mL) and 8.05 μL NHS (1 mg/mL) were added and the solution was incubated at room temperature for 30 min. 30 μL BSA-TTX (2.5 mg/mL) was then added and the solution was incubated at room temperature for 2 h. Next, 100 μL 10% BSA was added and allowed to sit for 1 h in order to block any excess carboxyls of the FM. Finally, the mixture was centrifuged at 10,706 RCF for 30 min and the precipitate was re-suspended in ultrapure water. For the preparation of FM-BSA, all steps were the same except that 30 μL BSA was added instead of BSA-TTX.

2.5. Fluorescence quenching immunochromatographic sensor (FQ-ICS)

2.5.1. Preparation of the FQ-ICS

FQ-ICS strips were prepared as follows: FM-BSA-TTX and FM-BSA were diluted 300-fold using 20 mM PB (pH 5.8) and were dispensed on separate areas of a NC membrane, designated as the test line (T-line) and calibration line (C-line), using an automatic dispenser delivering a volume of 1 μL/cm. To improve the combined efficiency between the AuNPs-mAb quencher and the FM-BSA-TTX on the T-line, 0.75 mg/mL BSA-TTX dissolved in PB (20 mM pH 5.8) was dispensed onto the T-line along with the FM-BSA-TTX. The sample pad was pretreated using PBS (0.015 M, pH 7.4, and