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A biolayer interferometry-based competitive biosensor for rapid and sensitive detection of saxitoxin

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ABSTRACT

Saxitoxin is a marine neurotoxin, which is commonly found as a paralytic shellfish toxin. It is characterized by strong toxicity, rapid effects and wide distribution with significant public health impacts. The ethical issues and technical defects associated with the currently applied surveillance system for paralytic shellfish toxins are encouraging further studies to develop suitable alternatives. Here, we report a label-free and real-time optical biolayer interferometry competitive biosensor for the detection of saxitoxin using aptamers as specific receptors. The biosensor exhibited a broad detection range from 10 to 2000 ng/mL of saxitoxin (linear range from 100 to 800 ng/mL), with a low detection limit of 0.5 ng/mL. Furthermore, the biosensor showed a high degree of selectivity for saxitoxin, and good reproducibility and stability with real samples. We believe that this novel biosensor offers a promising alternative to traditional analytical methods for the sensitive and rapid (7 min) detection of saxitoxin.
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1. Introduction

Saxitoxin (STX) is a hydrosoluble, heat-stable phycotoxin produced by both eukaryotic dinoflagellates and cyanobacteria [1]. STX has several analogues, such as neosaxitoxin (neoSTX), gonyautoxin 1/4 (GTX 1/4) and gonyautoxin 2/3 (GTX 2/3; Fig. 1a), which have very similar structures but different toxicities [2]. Accumulation by filter-feeding bivalves and fish, and the subsequent transfer through the food web, results in paralytic shellfish poisoning and even death [3]. The toxicity is thought to be caused by the ability of STX to bind to voltage-gated sodium channels, resulting in subsequent neural blockade and death via respiratory paralysis [4]. Since the contamination of shellfish with STX has adverse effects on human health and the global shellfish industry, detection methods for STX have been developed [5]. The mouse bioassay, standardized by the Association of Analytical Communities (AOAC), has been accepted internationally as the official method for the quantitative analysis of STX [6]. However, the assay suffers from cross-reactivity, low sensitivity, and ethical issues, which are considerable limitations. Some alternative analytical methods have been developed, such as high performance liquid chromatography (HPLC) with fluorescence or mass spectrometry, which has been adapted by the AOAC as an alternative for the detection of STX [7–9]. However, they are time-consuming, and require highly trained personnel as well as toxin reference standards, which are difficult to obtain. Therefore, the development of other methods is urgently required to achieve sensitive and rapid toxin monitoring.

Biosensors are ideal alternatives to traditional analytical methods for STX detection. Some optical immunosensors for STX detection based on surface plasmon resonance, fluorescence and electrochemistry have been developed, and demonstrate good sensitivity [10–12]. Nevertheless, a major drawback is the use of antibodies as molecular recognition elements. Antibodies possess a variety of limitations such as high production costs and cross-reactivity, special storage requirements, and the use of experimental animals [13]. Such drawbacks limit the widespread application of these immunosensors for STX detection. Aptamers are functional single-stranded DNA or RNA oligonucleotides selected from combinatorial libraries of random sequences by *in vitro* selection, as stable and low-cost recognition receptors that can replace antibodies [14–16]. Since the first aptamer to be identified in 1990, many aptamers have been obtained for a wide range

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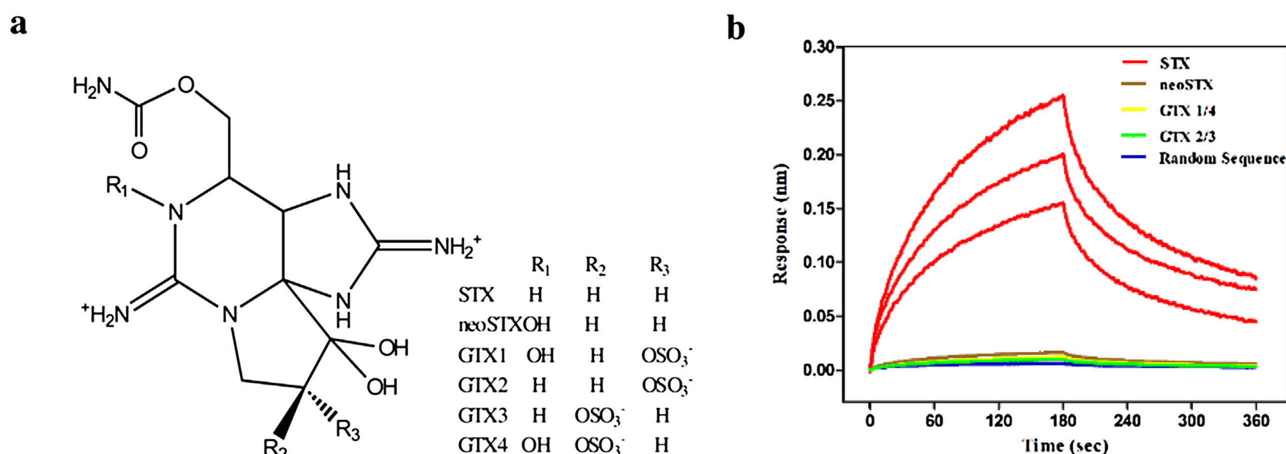

Fig. 1. (a) Chemical structures of STX, neoSTX, GTX 1/4 and GTX 2/3. (b) Assessment of affinity and specificity of the M-30f anti-STX aptamer for STX. The red lines represent the interaction curve of M-30f with STX (10 μM (top), 5 μM (middle), and 2.5 μM (bottom)). The blue line represents the interaction curve of a random sequence with STX. The gold, yellow, and green lines represent the interaction curves of M-30f with neoSTX, GTX 1/4 and GTX 2/3, respectively.

of target analytes, which has led to the development of various robust biosensing platforms [17]. In recent years, biolayer interferometry (BLI)-based biosensor has been rapid development, which can label-free and real-time optical detection of targets including small molecules, proteins, pathogens and cell [18–20]. We previously coupled aptamer with BLI technology and developed a rapid and sensitive biosensor for measuring of marine biotoxin [21].

In the present study, to accurately measure STX at lower concentrations, a competitive binding assay was established, between immobilized STX and free STX in solution in the presence of a fixed amount of the anti-STX aptamer (M-30f) [22]. The anti-STX aptamers were used as biorecognition receptors to competitively bind with STX, which was immobilized on the biosensor surface (Fig. 2a). This caused a large change in the optical thickness and mass density of the biosensor layer, shifted the interference pattern and generated the biosensor response (Fig. 2b). Indeed, aptamers, as recognition receptors, in conjunction with BLI technology and the use of an aptamer-based competitive binding assay, enables sensitive and high-speed detection of STX.

2. Experimental

2.1. All the materials, reagents, experimental methods and instrumentations used throughout this study are described in details in the supporting information

2.1.1. Biolayer interferometry assay

BLI is a label-free and real-time optical analysis technique that utilizes fiber-optic biosensors for measuring the interactions between biomolecules [23]. In this system (ForteBio, OctetRED96), the interaction of analytes with ligands immobilized on a sensor surface forms a monomolecular layer that in turn creates a proportional shift in the interference spectrum of reflected light [24]. This wavelength shift (Δλ, Fig. 2b) directly reflects the change in the optical thickness of the sensor layer, and is reported as a change in wavelength as a function of time. Any change in the number of molecules bound to the biosensor causes a shift in the interference pattern that can be measured in real-time. Therefore, the affinity and specificity of anti-STX aptamer M-30f were determined by BLI. The principle and analysis procedures used herein were as detailed in Concepcion et al. [25]. The assay process includes five steps: (1) baseline (2 min); (2) loading (5 min); (3) washing (2 min); (4) association (3 min); (5) dissociation (3 min). The response data obtained from the reaction surface were normalized by subtracting the signal simultaneously acquired from the reference surface to eliminate nonspecific binding and buffer-induced interferometry spectrum shift using the Octet Data Analysis Software CFR Part 11 Version 6.x. A 1:1 binding mode with mass transfer fitting was used to obtain the kinetic data.

3. Results and discussion

3.1. Assessment of the affinity and specificity of the anti-STX aptamer

In order to confirm that the M-30f anti-STX aptamer binds to STX with high affinity and specificity, a BLI assay was performed [26]. As shown in Fig. 1b, STX at different concentrations (10 μM, 5 μM, and 2.5 μM) was analyzed for association over 3 min and dissociation over 3 min, along with 10 μM neoSTX, GTX 1/4, and GTX 2/3 as non-specific targets as well as a blank sample containing only running buffer for reference. The results reveal that the M-30f aptamer interacts with STX with a kass (1/Ms) value of 7.40E + 04, a kdiss (1/s) value of 9.45E-03 and a kd (M) value of 1.28E-07. Moreover, M-30f was exposed to STX congeners, including neoSTX, GTX 1/4 and GTX 2/3, and no change in response was observed. A random sequence was used as an aptamer control and showed no binding to STX. These results demonstrate that the M-30f anti-STX aptamer bound with high affinity and specificity to STX.

3.2. BLI competitive biosensor

To achieve rapid and sensitive detection of STX, the anti-STX aptamer was then used to fabricate a label-free and real-time optical competitive biosensor based on BLI detection. As shown in Fig. 2a, competitive binding was established between the immobilized STX on the AR2G biosensor surface and free STX in the detection system, which was present with a fixed amount of the M-30f anti-STX aptamer. The detection of free STX was realized based on a change of response of the biosensor, mainly as a result of an increase in optical thickness at the biosensor surface. This results in a wavelength shift (Fig. 2b, Δλ) that is a direct measure of the change in thickness of the biological layer. With increasing concentrations of free STX in the detection system, the amount of the anti-STX aptamer which is available for binding to the immobilized STX on the biosensor surface is reduced, resulting in a lower detection signal. Theoretically, higher concentrations of the aptamer will result in decreased sensitivity and lower concentrations will result in a weak signal. Based on optimization of the experimental condi-

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