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Feasibility of using a surface plasmon resonance-based biosensor to detect and quantify yessotoxin

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ABSTRACT

Yessotoxin (YTX) is a disulfated polyether toxin produced by marine dinoflagellates. Although there is no clear evidence that YTX is toxic to humans, it is a major cause of false positives in DSP toxin detection by mouse bioassay. We developed a new detection and quantification method for yessotoxin using a BiaCore X Surface plasmon resonance (SPR)-based biosensor. The assay is based in the interaction of YTX with phosphodiesterase enzymes (PDE), one of its cellular targets. The injection of several YTX concentrations (3–12 μM) over immobilized PDE I, showed a dose dependent binding signal, which K_{obs} (observed rate constant) allowed us to obtain a calibration curve with a linear fit. The detection of yessotoxin using SPR-based biosensor allows the quantification of the toxin with an automated and repetitive method at concentrations in the range of the 1 mg kg^{-1} European regulatory limit.

1. Introduction

The increased occurrence of phycotoxins in coastal seawaters is a public concern, since they can pose a serious threat to human health and cause severe economic losses to aquaculture. One of these phycotoxins is yessotoxin (YTX, Fig. 1), which was first isolated from the hepatopancreas of the scallop *Patinopecten yessoensis* in Japan [1]. Nowadays yessotoxin is spread worldwide, having been described in Japan, Italy, Norway, New Zealand and Chile [2]. Initially, YTX was classified in the DSP toxin group because of its lipophilic nature and because it often coexists with these toxins [2]. Subsequent

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Abbreviations: SPR, surface plasmon resonance; DSP, diarrhetic shellfish poisoning; YTX, yessotoxin; PDE, phosphodiesterase; DMSO, dimethyl sulfoxide; PBS, phosphate-buffered saline; HBS-EP, hepes buffered saline; NHS, *N*-hydroxysuccimide; EDC, ethyl-N-(dimethylaminopropyl)carbodiimide; HPLC, high pressure liquid chromatography; LC–MS, liquid chromatography–mass spectrometry.

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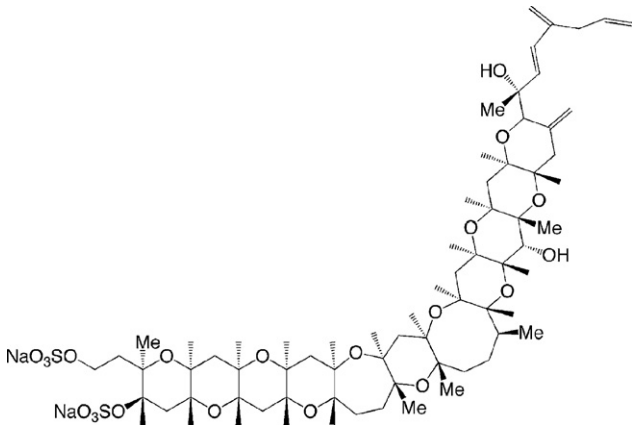

Fig. 1 – Yessotoxin structure.

studies demonstrated that YTX does not induce diarrhoea [3] and is not a PP2A inhibitor, a well-known target of DSP toxins [4]. Therefore, in many countries yessotoxin has been placed in a separate phycotoxin group for regulatory purposes, with a maximum permitted level of 1 mg of YTX equivalents kg^{-1} of shellfish [13].

Although yessotoxin toxicity to humans is unknown, oral administration to mice does not induce toxic effects [4]. However, its intraperitoneal lethality is the strongest among all lipophilic phycotoxins (0.1 mg kg^{-1}) [4] and its presence in shellfish may lead to a high overestimation of toxicity when the mouse bioassay is used for DSP toxin detection. Currently, YTX can be detected by several analytical methods, including HPLC with fluorimetric detection [5], LC–MS [6], enzyme-linked immunosorbent assays (ELISA) [7], fluorescence polarization [8] and resonant mirror-based biosensor [9]. In this work, we present a detection method using a surface plasmon resonance (SPR)-based biosensor. SPR-based biosensor detection offers the advantages of biomolecular interaction monitorization in real-time without the need of fluorescent labels and it is an automated system that improves accuracy and reliability versus other biosensors.

Although the exact mechanism of action of YTX is still unclear, the activation of cellular phosphodiesterases by this toxin has been recently described (PDEs) [10] and a direct interaction between YTX and PDEs was demonstrated using a resonant mirror biosensor (IAsys) [11]. Using this interaction, we developed a new and fast method for YTX detection and quantification in SPR-based biosensors (BiaCore X).

2. Materials and methods

2.1. Chemicals

YTX was purified by Dr. T. Yasumoto [1]. CM5 sensor chips, HBS-EP (Hepes buffered saline) and amine coupling kit were supplied by BiaCore AB (Uppsala, Sweden). HCl was purchased from Panreac Quimica SA (Barcelona, Spain). Phosphodiesterase I (PDE I, type IV from *Crotalus atrox*), phosphodiesterase 3′,5′-cyclic-nucleotide-specific (PDEs, from bovine brain), dimethyl sulfoxide (DMSO), sodium acetate and Tween-20 were from Sigma Chemical Co. (Madrid, Spain). Phosphate buffered saline solution (PBS/T) was: 8.2 mM Na_2HPO_4, 137 mM NaCl, 1.5 mM KH_2PO_4, 3.2 mM KCl, pH 7.4, plus 0.05% Tween-20 (Sigma). PBS/T was degassed and filtered through a Millex® GP 0.22 μm pore size filter from Millipore Corporation (Bedford, MA, USA). PB was: 8.5 mM Na_2HPO_4, 1.5 mM NaH_2PO_4, pH 7.7.

2.2. PDE immobilization

Sensor surface activation and ligand immobilization were performed using PBS/T as running buffer at a flow rate of 5 μL min^{-1} and 25 °C. The activation of the CM5 dextran matrix was carried out by injection over the chip surface of a freshly prepared mixture of equal volumes of 75 mg mL^{-1} EDC and 11.5 mg mL^{-1} NHS for 10 min. PDE I or PDEs were dissolved in 10 mM acetate buffer, pH 5.0 at a final concentration of 0.25 mg mL^{-1}, and injected over the chip for 14 min. Deactivation of the remaining NHS active esters was achieved by injection of 1 M ethanolamine-HCl, pH 8.5, for 5 min. After injection of 10 mM HCl for 1 min, the surface was ready for binding experiments (supplementary material Figure 4S).

2.3. Association experiments and chip regeneration

The interaction of YTX with immobilized phosphodiesterase was monitored at 25 °C, using HBS-EP as running buffer at a flow rate of 10 μL min^{-1}. Serial dilutions of yessotoxin stock (5 mM in DMSO) were prepared in HBS-EP at 3, 5, 7, 10 and 12 μM concentrations and injected for 2 min. Control solutions with a matching concentration of DMSO were injected in the same way and subsequently subtracted from YTX response signal. Regeneration of the surface between injections was carried out with a single 1 min pulse of 10 mM HCl.

Since YTX dissociates almost completely, we used the association phase to quantify the YTX-PDE I interaction. A pseudo-first-order association rate constant K_{obs} (observed rate constant) was determined using the 1:1 Langmuir association model of the BiaEvaluation software (BiaCore), from the binding data of each toxin concentration.

3. Results and discussion

Two different phosphodiesterases, PDE I from *C. atrox* and PDEs from bovine brain were assayed to determine the better ligand for yessotoxin in our conditions. Both PDE I or PDEs activated surfaces showed binding activity after injection of YTX. However, at low yessotoxin concentrations, the PDE I sensor chip had a higher sensitivity and therefore the PDE I-CM5 chip was used for further assay optimization. Two running buffers were tested for optimum binding: a phosphate buffer (PB) and a HEPES buffered saline (HBS-EP). Binding of YTX to the PDE I chip was lower in PB than in HBS-EP. The regeneration solution (10 mM HCl) allowed for a complete regeneration of the sensor surface with retention of binding activity at least for 50 cycles of regeneration.

The injection of several YTX concentrations (3–12 μM) over the PDE I activated chip showed a dose dependent binding signal (Fig. 2). The interaction between YTX and PDE I seemed

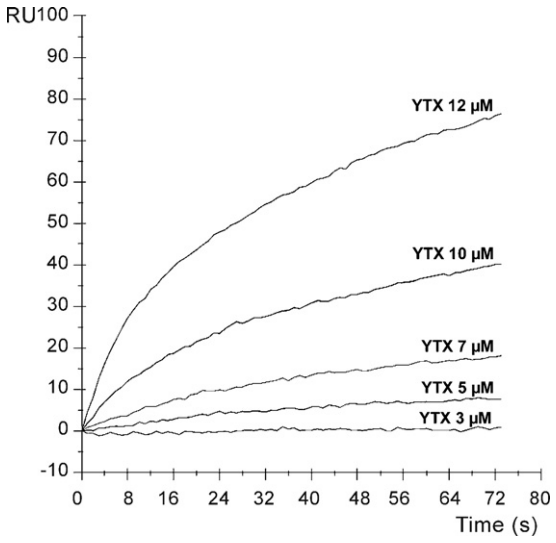

Fig. 2 – Association curves of YTX to immobilized PDE I. YTX was injected over the PDE I-CM5 chip at concentrations of 3, 5, 7, 10 and 12 µM using HBS-EP as running buffer and a flow rate of 10 µL min^{-1}. The association curves of all YTX concentrations were obtained after subtraction of their respective DMSO controls. The time interval from 7 to 81 s used for K_{obs} calculation is represented. Representative of 4 experiments.

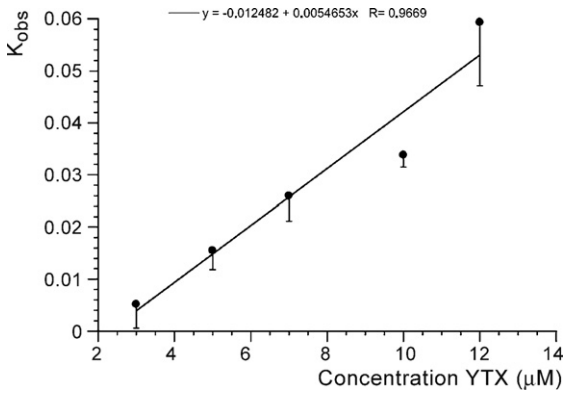

Fig. 3 – Yessotoxin calibration curve. YTX was injected over the PDE I-CM5 chip at concentrations of 3, 5, 7, 10 and 12 µM. The K_{obs} of the association curves (e.g. Fig. 2) was calculated by the BiaEvaluation software and plotted versus toxin concentration (mean ± S.E.M., $n = 4$).

to be reversible, showing a fast, almost complete dissociation after toxin removal (supplementary material Figure 2S-A). This characteristic of the interaction, together with the high bulk effect due to the toxin carrier (DMSO) (supplementary material Figure 2S-A and 2S-B), prevented us from working with absolute binding signal. Since the YTX-PDE I interaction follows a pseudo-first-order kinetics, the K_{obs} of the binding curve was used to quantify YTX. The K_{obs} was calculated by the BiaEvaluation software (BiaCore) between 7 and 81 s after the toxin injection was started. K_{obs} was plotted versus YTX concentration, obtaining a calibration curve with a linear fit, $y = 0.00546x - 0.01248$, $R = 0.9669$, for $n = 4$ experiments (Fig. 3). The range of quantification of this SPR-biosensor assay is similar to that reported previously for the resonant mirror biosensor assay, which allowed for quantification of the toxin around the levels of the current European regulatory limit (1 mg of YTX kg^{-1} of whole body or any part edible) using an acetone extraction with posterior liquid/liquid partition in dichloromethane/H_2O [9]. Moreover, PDE I binds several YTX derivatives [12], susceptible of detection by this assay. Although the assay sensitivity for YTX is similar in both biosensors, the SPR-based biosensor assay has several advantages versus the resonant mirror biosensor assay. Besides being ten times faster than the previous method developed in a resonant mirror-based IAsys biosensor (contact time of the sample was reduced from 20 to 2 min), the SPR-biosensor assay accuracy and reproducibility is improved by the constant flow and semiautomatic injection technologies incorporated in the BiaCore X biosensor.

In summary, this paper reports a new, sensitive and fast assay to detect YTX in the range of the European regulatory limit, considerably improving previously reported detection with biosensor technologies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.aca.2008.01.010.

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