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Developing improved immunoassays for paralytic shellfish toxins: The need for multiple, superior antibodies

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ABSTRACT

Paralytic shellfish toxins (PSTs) are a risk to humans upon consumption of contaminated seafood. The PST family is comprised of more than twenty congeners, with each form having a different potency. In order to adequately protect consumers yet reduce unnecessary closures of non-contaminated harvesting areas, a rapid method that allows for analysis of sample toxicity is needed. While a number of PST immunoassays exist, the outstanding challenge is linking quantitative response to sample toxicity, as no single antibody reacts to the PST congeners in a manner that correlates with potency. A novel approach, then, is to combine multiple antibodies of varying reactivity to create a screening assay. This research details our investigation of three currently available antibodies for their reactivity profiles determined using a surface plasmon resonance biosensor assay. While our study shows challenges with detection of the R1-hydroxylated PSTs, results indicate that using multiple antibodies may provide more confidence in determining overall toxicity and the toxin profile. A multiplexed approach would not only improve biosensor assays but could also be applied to lateral flow immuno-chromatographic platforms, and such a theoretical device incorporating the three antibodies is presented. These improved assays could reduce the number of animal bioassays and confirmatory analyses (e.g., LC/MS), thereby improving food safety and economic use of shellfish resources.

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1. Introduction

Paralytic shellfish poisoning (PSP) is caused by a suite of toxins, known collectively as paralytic shellfish toxins (PSTs) [1]. Saxitoxin (STX) and its congeners originate from certain dinoflagellates and some cyanobacteria [2]. Filter feeding bivalves (e.g., mussels, clams, cockles, scallops and oysters), as well as other seafood species, can accumulate and metabolize these toxins which can then lead to potentially dangerous seafood [3,4]. Human consumption of toxic seafood can result in tingling, numbness, respiratory paralysis and potentially death [5], as the PSTs bind to site 1 and block the opening of voltage gated sodium channels [6]. These small molecule toxins are also quite robust, and typical preventative food safety measures (i.e., use of heat or acid during cooking) do not destroy the PSTs [1].

Proper monitoring and implementation of harvesting bans when toxin concentrations exceed safe levels (typically 80 μg STX equivalents per 100 g tissue) have minimized PSP illnesses [1]. However, outbreaks still occur, especially in developing countries [7] and with an estimated worldwide mortality of 6% [8]. For example, a major PSP epidemic occurred in Guatemala in 1987 that claimed the lives of 26 people out of the 187 affected [7,9]. A review of PSP cases and outbreaks has been compiled by FAO, which reports PSP prevalence along coastal European nations, parts of Africa, the West Coast and Northeast region of North America, South America, and parts of Asia [10]. Within the US, the majority of illnesses and outbreaks are reported from recreational harvests among fishermen and tribal communities. For example, during May and June of 2011, 21 cases of PSP illness were reported in Southeast Alaska due to unprecedented high levels of PSTs in surrounding waters [11].

Recent reviews on PST detection have focused on improved analysis of both coastal waters and seafood [1,2,12,13]. The mouse bioassay (MBA) is one of the AOAC approved and most commonly used testing methods for PSTs [14]. While simple, this bioassay suffers performance related challenges (e.g., poor quantitation and low dynamic range, interferences to detection, low sample throughput, and lack of determination of the specific toxin associated with death) as well as ethical concerns.

A second AOAC approved method for determining PSTs is high performance liquid chromatography (HPLC) with fluorescence detection (FD) [15,16]. This method is quite effective at identifying and quantifying the toxins in a seafood sample. However, it requires a lengthy sample clean-up and pre-column oxidation procedure to

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create fluorescent derivatives of the toxins for detection as well as multiple analytical runs for complete PST determination. The post-column HPLC-FD method created by Oshima [17] was refined [18] and is also now AOAC approved [19]. This post-column oxidation method has a simpler sample preparation procedure than pre-column HPLC-FD; however, multiple analytical runs under different chromatographic conditions must be conducted in order to analyze all potential PST congeners. Furthermore, both HPLC-FD approaches can be hindered by sample materials that have native fluorescence, requiring additional steps to ensure the presence of toxins [20].

Other analytical techniques that are advancing include liquid chromatography (LC) coupled with mass spectrometry (MS) [21,22], some in tandem with biosensors [23]. The major limitation of this analytical approach is matrix interference and ionization suppression, which restricts its ability to serve as a reliable, quantitative monitoring tool. Limited availability of internal reference standards (e.g., isotopically labeled toxins) currently hinders wider-spread implementation of monitoring by LC/MS.

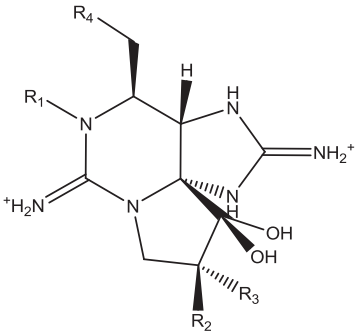
In order to overcome the challenges associated with MBA and LC methods, rapid screening techniques have been explored. These methods can be simple, cost-effective, sensitive, and accurate for high-throughput detection needs. Such methods include receptor binding assays (RBA) [24–27], lateral flow immuno-chromatography [28,29], enzyme-linked immunosorbent assays (ELISA) [30–32], and cell bioassays [33,34]. While these methods allow for high throughput and ease of use, they suffer from the use of difficult to procure radiolabeled materials for RBA, high probability of false-positive and potential for false-negative results with current immuno-chromatographic PSP tests, large amounts of manual labor and limited antibody cross-reactivity for ELISA, and nonspecific toxin recognition for the cell bioassays.

An immunological technique that has been shown to provide high throughput detection of PSTs is surface plasmon resonance (SPR) biosensors [35–38], though this method faces the same challenges with respect to antibody reactivity. SPR immunoassays are based on specific biosensor platforms that bind the molecule of interest at the surface. The change in mass due to binding is detected as a change in refractive index (RI) at the dielectric interface (i.e., gold immunoassay substrate and solution in the flow cell). This RI change causes a shift in the SPR band position that can be tracked in real-time using standard spectroscopy optics [39]. This automated technique allows for real-time analysis of PST-containing samples, requires minimal sample cleanup, no labeling of the analytes, and yields sub-ppb limits of detection in less than ten min [40,41].

The SPR assay for the determination of PSTs currently implemented in our laboratory is robust and shows good repeatability and reproducibility; however, quantitative results do not always correlate with overall sample toxicity due to the many PST congeners having widely varying potency. The toxicities for common PSTs are shown in Table 1, and the inability to correlate results with sample toxicity when using immunological assays could lead to unsafe seafood harvested for consumers (false-negative) or destruction of safe seafood and closure of non-contaminated harvesting areas (false-positive). Clearly, there is a need for improved assays to not only protect the public but also to improve the economic viability of the industry and utilization of seafood resources. Unfortunately, a single antibody that reacts to the congeners with respect to their potency has yet to be produced. An advantage to the SPR assay is that while the response may not always correlate with toxicity, the cross-reactivity of individual congeners with an antibody can be calculated. A novel approach, then, would be to combine multiple antibodies of varying reactivity to the congeners, as screened via the SPR assay, to create a multiplexed immunoassay.

One disadvantage to SPR biosensors is the size of instrumentation and cost of materials which could prohibit routine testing in the field or dockside. Lateral flow immuno-chromatographic tests (LFIs) have been used for PST testing and could fulfill the requirements of an easy-to-use and cost-effective technique for monitoring potential toxicity of seafood when the quantitation and automation of the SPR instrumentation is not necessary.

Table 1
PST structure, congener forms, and relative toxicities [17]. Toxins used in this study are listed in bold.



Toxin	Relative Toxicity
STX	1.00
dcSTX	0.51
GTX2,3	0.36, 0.64
B1 (GTX5)	0.06
C1,2	0.01, 0.10
dcGTX2,3	0.15, 0.38
NEO	0.92
dcNEO	–
GTX1,4	0.99, 0.73

R1	R2	R3	Carbamate	Decarbamoyl	*N*-sulfocarbamoyl
H	H	H	**STX**	**dcSTX**	**B1 (GTX 5)**
OH	H	H	**NEO**	**dcNEO**	B2 (GTX 6)
OH	H	OSO_3^-	**GTX1**	dcGTX1	C3
H	H	OSO_3^-	**GTX2**	**dcGTX2**	**C1**
H	OSO_3^-	H	**GTX3**	**dcGTX3**	**C2**
OH	OSO_3^-	H	**GTX4**	dcGTX4	C4
		R4: