CONffIDENCE: Contaminants in food and feed: Inexpensive detection for control of exposure

Multiplex based analysis for marine biotoxins

K. Campbell [1]; J. Buijs [2]; A.C. Huet [3]; N. Vilariño [4]; L.M. Botana [4]; C.T. Elliott [1]

[1] Institute of Agri-Food and Land Use, School of Biological Sciences, Queen's University, David Keir Building, Stranmillis Road, Belfast, United Kingdom. BT9 5AG

[2] GE Healthcare Bio-Sciences, Rapsgatan 23, 75184 Uppsala, Sweden

[3] Centre d'Economie Rurale (CER Groupe), Département Santé, rue Point du Jour 8, 6900 Marloie, Belgium.

[4] Departamento de Farmacología, Facultad de Veterinaria, Universidad de Santiago de Compostela, Campus Universitario, 27002 Lugo, Spain

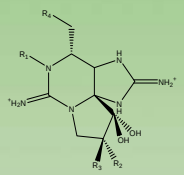
Introduction

Despite ethical concerns, for phycotoxin analysis in shellfish the reference method of analysis remains as the biological method or more commonly referred mouse bioassay. This is particularly the case for the global monitoring of diarrheic (DSP) and paralytic shellfish poisoning (PSP) toxins. The detection of the amnesic shellfish poisoning (ASP) toxin domoic acid is carried out by HPLC analysis and in recent years a laborious HPLC method has been developed for PSP toxin analysis. In FP6 several projects (Biotox, Biotoxmarin, Detectox, BioCop) were funded in the field of marine biotoxin analysis. Surface plasmon resonance (SPR) technology was displayed as a highly promising immunoassay based tool since it offers rapid real time detection requiring minimal reagents and standards due to the microfluidic system. The latter being essential because of the limited availability and supply of toxin standards. Hence, alternative methods of detection to the biological methods have been described but to date each analytical method is specific for individual toxins and their immediate analogues with each group of toxins requiring individual tests. The ideal scenario for the monitoring of marine biotoxins would be to incorporate as many of the toxins onto a single assay format - one test fits all.

Aim

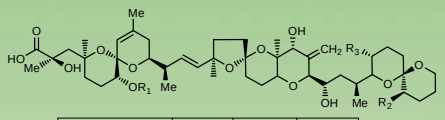
The aim of this research was to develop a multiplex SPR biosensor assay capable of detecting a combination of PSP/DSP/ASP toxins. The major toxin targets to be detected in shellfish tissue for each group are:

PSP: Saxitoxin (STX), neosaxitoxin (NEO), gonyautoxin (GTX) 1/4,



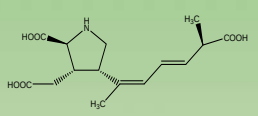
			Carbamate Toxins	*N*-Sulfo carbamoyl toxins	Decarbamoyl toxins	Deoxy decarbamoyl toxins
R_1	R_2	R_3	R_4: $OCONH_2$	R_4: $OCONHSO_3^-$	R_4: OH	R_4: H
H	H	H	STX	B1 (GTX 5)	dc-STX	do-STX
H	H	OSO_3^-	GTX 2	C1	dc-GTX 2	do-GTX 2
H	OSO_3^-	H	GTX 3	C2	dc-GTX 3	do-GTX 3
OH	H	H	NEO	B2 (GTX 6)	dc-NEO	
OH	H	OSO_3^-	GTX 1	C3	dc-GTX 1	
OH	OSO_3^-	H	GTX 4	C4	dc-GTX 4	

DSP: Okadaic acid (OA), DTX1-2



Toxin	R_1	R_2	R_3
Okadaic Acid	H	H	H
DTX-1	H	CH_3	CH_3
DTX-2	H	CH_3	CH_3

ASP: Domoic acid (DA)

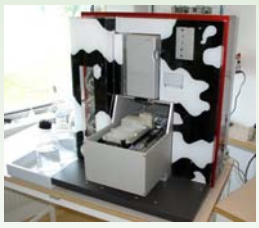


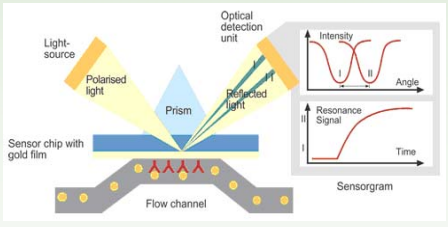
Methods

Multiplex Biosensor Analysis

Optical surface plasmon resonance (SPR) biosensors are a fast and label-free alternative to classical binding assays such as ELISA, RIA and RRA. Their scope is limited by the cross-reactivity of the bio-recognition element. However, multiplexing can be achieved by the development of multi-flow cell SPR and/or by the development of multi-spots within a single SPR flow cell for different bio-recognitions. Within the scope of FP6 project BioCop a prototype 16-plex (4 spots x 4 flow cells) SPR instrument was developed for the detection of protein biomarkers. This device is a molecular interaction array system that can perform concentration analysis of multiple analytes.

Within CONffIDENCE this technology was applied to multiplex marine biotoxin analysis. A toxin assay was incorporated into each of the four flow cells of a single biosensor chip thus allowing samples to be analyzed under different cycle conditions.

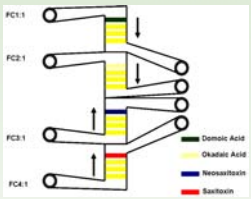




These toxin assays are performed using an indirect inhibition assay format, in which specific antibodies are mixed with standards / shellfish samples and run over a chip with immobilised target toxins. No toxin present response is high, toxin present response is low.

Chip Surface Immobilisation

The chip surface immobilization unit was designed with 16 isolated microfluidic channels for the immobilization of up to 16 different ligands on a chip surface. When docked, the sensor then forms 4 microfluidic flow cells with 4 immobilised spots with each.

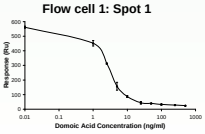


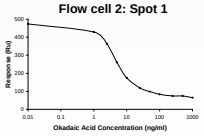
For the immobilisation of each toxin an amine surface was prepared on the appropriate spot. Domoic acid and okadaic acid were conjugated to each amine surface spot using carbodiimide chemistry whereas neosaxitoxin and saxitoxin were conjugated using a modification of the Mannich reaction.

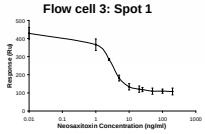
Each flow cell was assessed using the antibodies available at QUB for each of the toxin targets. Each antibody was mixed in a 1 to 1 ratio with toxin standards to produce calibration curves. The mix was then injected over the chip surface at a flow rate of 20µl/min with a contact time of 180s. The regeneration solutions were optimised for each antibody toxin combination. The flow rate for the regeneration solution was also set at 20µl/min and the contact time at 30s followed by a 90s stabilisation time prior to the next injection.

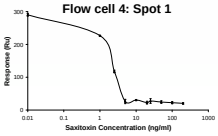
Results

Calibration Curves were produced on each flow cell for the specified toxin









Toxin	Antibody	Titre	IC_{50} (ng/ml)	$IC_{20} - IC_{80}$ (ng/ml)
Domoic Acid	R867	1/200	2.6	1.0 – 6.4
Okadaic Acid	R739	1/4000	4.9	1.7 – 14.4
Neosaxitoxin	NEO-Ab	1/25	2.6	1.1 – 6.0
Saxitoxin	R895	1/1000	1.9	1.0 – 3.7

Toxin	Regeneration solution
Domoic Acid	75mM sodium hydroxide
Okadaic Acid	180mM sodium hydroxide with 15% acetonitrile
Neosaxitoxin	100mM Hydrochloric Acid
Saxitoxin	50mM Hydrochloric Acid

Conclusions

A single multiplex chip was produced for the simultaneous detection of PSP/DSP/ASP toxins.

Each antibody / toxin combination was isolated within a different flow cell whereby different regeneration solutions could be applied.

A prototype biosensor assay was developed for the key toxins and calibration curves produced.

Future Work

The next step is to provide a single simple, rapid and reliable sample preparation that is compatible for all the target toxins.

The discovery of two new classes of potent and fast acting toxins, spirolides and palytoxins, has given strong impetus to assess their toxicity and inclusion within this rapid test format.

The research leading to these results has received funding from the European Community's Seventh Framework Programme (*FP7/2007-2013)* under *grant agreement* n° 211326: CONffIDENCE project (www.conffidence.eu).