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Dynamics of paralytic shellfish toxins and their metabolites during timecourse exposure of scallops *Chlamys farreri* and mussels *Mytilus galloprovincialis* to *Alexandrium pacificum*

Jiangbing Qiu[a], Fanping Meng[a,b], Ling Ding[a], Yijia Che[a], Pearse McCarron[c], Daniel G. Beach[c], Aifeng Li[a,b,*]

[a] College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China
[b] Key Laboratory of Marine Environment and Ecology, Ocean University of China, Ministry of Education, Qingdao 266100, China
[c] Measurement Science and Standards, National Research Council Canada, 1411 Oxford St, Halifax, NS, B3H 3Z1, Canada

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ABSTRACT

New *C*-11 hydroxyl metabolites of paralytic shellfish toxins (PSTs) have been reported in shellfish. To gain further information on these metabolites, as well as the potential for formation of phase-II metabolites and acyl esters of PSTs, bivalves were fed with the PSTs-producing dinoflagellate *Alexandrium pacificum* (strain ATHK). Through independent experiments, scallops (*Chlamys farreri*) were fed for 9 days and mussels (*Mytilus galloprovincialis*) for 5 days plus an additional 5 days of depuration, with representative samples taken throughout. Several common PSTs (C1-4, GTX1-6 and NEO) and metabolites including M1, M3, M5, M7, M9, M2 and M8 were detected in the hepatopancreas of scallops during toxin accumulation and in the hepatopancreas of mussels during both toxin accumulation and elimination periods. The relative molar ratio of metabolites to precursor molecules was used to estimate relative metabolic conversion rates. Conversion rates of C1/2 and GTX2/3 were higher than those of C3/4 and GTX1/4, in scallops and mussels. The first metabolites observed in both bivalve species investigated were M1/3, which are formed from C1/2. However, the conversion of GTX2/3 to M2 was more complete than other biotransformation reactions in both mussels and scallops. In general, metabolic conversion of PSTs was observed after a shorter time and to a greater extent in mussels than in scallops in the exposure period. No acyl esters or conjugation products of PSTs with glucuronic acid, glutathione, cysteine and taurine were detected by liquid chromatography with high resolution tandem mass spectrometry in the samples investigated. Additionally, only GTX1/4 and GTX2/3 were detected in the kidney of scallops, which demonstrates that PSTs are mainly metabolized through the hepatic metabolism pathway in bivalves. This work improves the understanding of PST metabolism during toxin accumulation and depuration in commercially harvested shellfish.

1. Introduction

Paralytic shellfish poisoning is a serious seafood safety issue resulting from the consumption of shellfish contaminated with paralytic shellfish toxins (PSTs). Paralytic shellfish toxins can cause seafood-borne illness or even death due to their acute neurotoxicity. More than 50 analogues have been reported (Wiese et al., 2010) and can generally be divided into three groups including carbamate (STX, NEO, GTX1-4), decarbamoyl (dcSTX, dcNEO, dcGTX1-4), and *N*-sulfocarbamoyl (GTX5-6, C1-4) toxins. Table 1 shows the structures of some common analogues and more recently reported metabolites of PSTs (Dell'Aversano et al., 2008; Quilliam et al., 2017). Marine dinoflagellates of the *Alexandrium*, *Gymnodinium* and *Pyrodinium* genera and several strains of freshwater cyanobacteria are known to produce PSTs. Commercially harvested bivalve shellfish can feed on these PSTs-producing dinoflagellates and accumulate high levels of toxins (Fernández-Reiriz et al., 2008). As a result, PSTs contaminated seafood poses a serious problem for the aquaculture industry and human health and regulatory monitoring programs are in place worldwide to help avoid human intoxication (Turner et al., 2008, 2010).

Numerous studies have been carried out to investigate the accumulation, transformation and elimination of PSTs in scallops (Shimizu and Yoshioka, 1981; Choi et al., 2003; Estrada et al., 2007; Kaga et al., 2015), mussels (Blanco et al., 2003; Kwong et al., 2006; Botelho et al.,

* Corresponding author at: College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China.
E-mail address: lafouc@ouc.edu.cn (A. Li).

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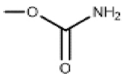
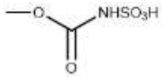
Table 1
Chemical structure of common analogues and new metabolites of PSTs (Quilliam et al., 2017).

Ring Structure	Toxin	Ring	R1	R2	R3	R4
A	Carbamate toxins					
	STX	A	H	H	H	
	NEO	OH	H	H		
	GTX1	A	OH	H	OSO_3^-	
	GTX2	A	H	H	OSO_3^-	
	GTX3	A	H	OSO_3^-	H	
	GTX4	A	OH	OSO_3^-	H	
	M2	A	H	OH	H	
	M4	A	H	OH	OH	
	M6[a]	B	H	OH	OH	
	M8[a]	A	OH	OH	H	
	M10[a]	A	OH	OH	OH	
	N-sulfocarbamoyl toxins					
	GTX5	A	H	H	H	
	GTX6	A	OH	H	H	
B	C1	A	H	H	OSO_3^-	
	C2	H	OSO_3^-	H	H	
	C3	A	OH	H	OSO_3^-	
	C4	A	OH	OSO_3^-	H	
	M1	A	H	OH	H	
	M3	A	H	OH	OH	
	M5[a]	B	H	OH	OH	
	M7[a]	A	OH	OH	H	
	M9[a]	A	OH	OH	OH	
	Decarbamoyl toxins					
	dcSTX	A	H	H	H	−OH
	dcNEO	A	OH	H	H	
	dcGTX1	A	OH	H	OSO_3^-	
	dcGTX2	A	H	H	OSO_3^-	
	dcGTX3	A	H	OSO_3^-	H	
	dcGTX4	A	OH	OSO_3^-	H	

[a] Structure from Quilliam et al. (2017).

2010; Kim and Shin, 2015), clams (Murakami et al., 1999; Samsur et al., 2006; Botelho et al., 2012; Turner et al., 2013), oysters (Murray et al., 2009; Guéguen et al., 2011; Xie et al., 2013) and cockles (Botelho et al., 2015; Costa et al., 2016). Results reported previously show that these bivalve species accumulate and eliminate PSTs differentially. For example, mussels can accumulate high levels of toxins in a short time-period (about 2 weeks) but also eliminate them quickly (Shumway, 1990). However, the accumulation and detoxification rates of PSTs in scallops are generally slower than those in mussels, taking up to several months to eliminate PSTs (Bricelj and Shumway, 1998). The viscera of bivalves are the main repository of PSTs during uptake and depuration (Estrada et al., 2007), accounting for 80 − 98% of the total toxicity (Bricelj and Shumway, 1998). Meanwhile, different analogues of PSTs are selectively biotransformed and eliminated in bivalve tissues to some degree (Tan and Ransangan, 2015). Metabolism of PSTs by shellfish includes desulfation, oxidation, reduction and hydrolysis (Oshima, 1995; Turner et al., 2013). This can include epimerization of the less stable β-epimers (C2, GTX3, GTX4) to α-epimers (C1, GTX1, GTX2), conversion of carbamate toxins to decarbamoyl toxins (Choi et al., 2003) or the reduction of the *N*-1 hydroxyl group of carbamate toxins (Oshima, 1995).

Recently, several new derivatives of saxitoxin (M1-M5) were discovered in mussels following an intensive bloom of *Alexandrium* spp. (Dell'Aversano et al., 2008). These new analogues (named M-toxins) were considered metabolites in shellfish, because they were not detected in the microalgae. Additional compounds (M6-M10) have been detected, with similar structural characteristics of single or double hydroxyl groups at *C*-11 (Table 1) (Quilliam et al., 2017). There have now been a number of reports of field contaminated shellfish such as mussels, clams, cockles and scallops, containing these metabolites (Vale, 2010; Li et al., 2012; Ding et al., 2017). Recently, biotransformation pathways for these new metabolites in shellfish were proposed (Ding et al., 2017). M1/M3 and M7/M9 are transformed from C2 and C4, respectively. M2, M4, M6 are likely converted from GTX2/3, while M8 and M10 appear to be derived from GTX1/4.

It is currently unknown whether other metabolites of PSTs such as phase-II metabolites or acyl esters, can be formed in shellfish. The phase-II conjugation of PSTs with glucuronic acid and glutathione (GSH) were reported during *in vitro* experiments in vertebrates (García et al., 2009, 2010; Sato et al., 2000). The glucuronidation reaction of STX, NEO and GTX2/3 occurred *in vitro* in human liver microsomes (García et al., 2010). GSH and cysteine were also reported to mediate the metabolism of PSTs in shellfish (Oshima, 1995; Lin et al., 2004). A range of acyl esters of other phycotoxin groups are known to form as metabolites in shellfish, such as for okadaic acid, dinophysistoxins-1, 2 (Marr et al., 1992; Doucet et al., 2007; Torgersen et al., 2008), brevetoxins (Morohashi et al., 1995), pectenotoxins (Wilkins et al., 2006), spirolides (Aasen et al., 2006), pinnatoxins (McCarron et al., 2012) and gymnodimines (de la lglesia et al., 2013). These phycotoxins have hydroxyl groups that can be acylated with fatty acids. Paralytic shellfish toxins also contain hydroxyl groups, however it is unknown whether similar acyl esters of PSTs occur in shellfish.

In the present study, a time-course exposure of scallops (*Chlamys farreri*) and mussels (*Mytilus galloprovincialis*) to the PSTs-producing dinoflagellate *A. pacificum* was independently carried out under laboratory conditions to more comprehensively investigate the metabolism of PSTs. Targeted MS/MS and untargeted HRMS approaches were used to search for reported and hypothesized metabolites of PSTs that could be present in shellfish.

2. Methods and materials

2.1. Chemicals

Certified reference materials of STX, NEO, C1/2, GTX1/4, GTX2/3, GTX5, dcSTX and dcGTX2/3 were obtained from National Research