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Profiles and levels of fatty acid esters of okadaic acid group toxins and pectenotoxins during toxin depuration, Part I: Brown crab (*Cancer pagurus*)

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ABSTRACT

In 2002, two outbreaks of diarrhetic shellfish poisoning (DSP) occurred in Norway, which was later confirmed to be caused by the consumption of brown crab (*Cancer pagurus*) contaminated predominantly by esters of okadaic acid (OA) after feeding on toxic blue mussels (*Mytilus edulis*). In addition to OA-group toxins, pectenotoxins (PTXs) are commonly detected in the toxin-producing algae (i.e. *Dinophysis*). In this paper, an experiment was set up to study the fatty acid ester profiles and depuration rates of OA-group toxins and PTXs from *C. pagurus* after feeding on *M. edulis* containing these toxin groups. OA, DTX1, DTX2 and PTX2 SA were all detected primarily in the form of fatty acid esters in the crab hepatopancreas (HP). Crabs preferentially assimilated toxins of the OA group after feeding on the mussels for 1 week. Detailed analysis of the fatty acid ester profile in crabs and mussels showed that the ester profiles in the crabs differed slightly from profiles of the fatty acid esters in *M. edulis*, but neither ester profile nor ester to free toxin ratio appeared to change in the crabs during the first 2 weeks of depuration. Calculations of depuration rates of the free forms of toxins resulted in similar reduction rates for OA and DTX2, whereas the depuration rate of DTX1, PTX2 and PTX2 SA was considerably faster. From the industrial perspective, the PTX-compounds are of minor importance compared to the OA group toxins in crabs, considering (1) the uncertainty regarding the oral toxicity of the PTXs, (2) the preferential ingestion of OA-group toxins compared to PTXs and (3) the faster depuration of PTXs.

1. Introduction

Filter-feeding bivalve molluscs, such as mussels, cockles, scallops and oysters, are the major vectors for shellfish poisoning syndromes in man (Shumway, 1995). The causative agents originate from various phytoplankton species, producing a diverse range of secondary metabolites toxic to humans (Daranas et al., 2001). Marine algal toxins associated with shellfish have traditionally been divided into different groups depending on the symptoms they introduce, like Paralytic Shellfish Poisoning (PSP), Diarrhetic Shellfish Poisoning (DSP), Neurotoxic Shellfish Poisoning (NSP) and Amnesic Shellfish Poisoning (ASP). Recently, re-grouping of the toxins based on chemical characteristics was suggested by an FAO/IOC/WHO working group, dividing them into eight toxin groups; azaspiracid group, brevetoxin group, cyclic imine group, domoic acid group, okadaic acid group, pectenotoxin group, saxitoxin group and yessotoxin group (Toyofuku, 2006).

The okadaic acid (OA) group of toxins (Fig. 1) consists of okadaic acid (OA) and its analogues dinophysistoxin-1 (DTX1) and dinophysistoxin-2 (DTX2), which are detected

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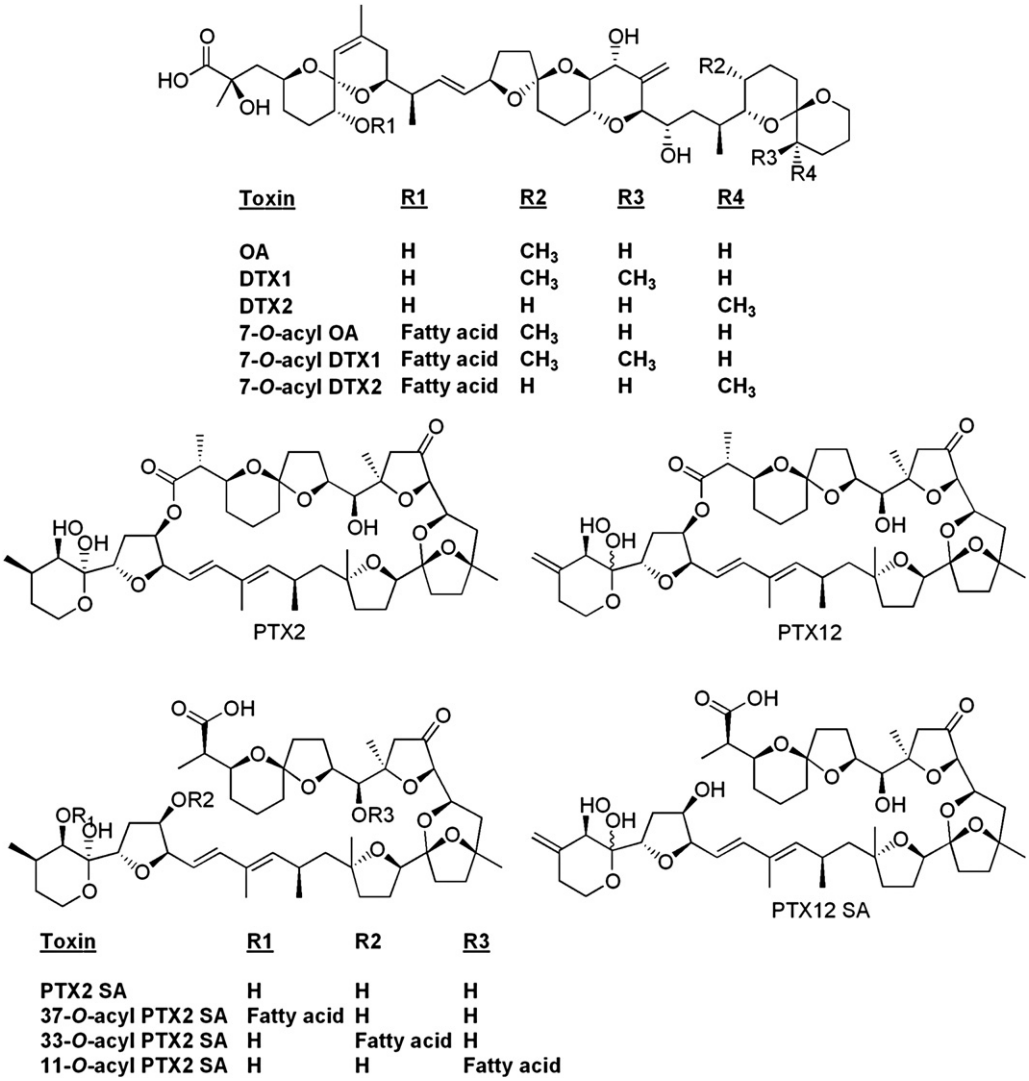

Toxin	R1	R2	R3	R4
OA	H	CH_3	H	H
DTX1	H	CH_3	CH_3	H
DTX2	H	H	H	CH_3
7-*O*-acyl OA	Fatty acid	CH_3	H	H
7-*O*-acyl DTX1	Fatty acid	CH_3	CH_3	H
7-*O*-acyl DTX2	Fatty acid	H	H	CH_3

Toxin	R1	R2	R3
PTX2 SA	H	H	H
37-*O*-acyl PTX2 SA	Fatty acid	H	H
33-*O*-acyl PTX2 SA	H	Fatty acid	H
11-*O*-acyl PTX2 SA	H	H	Fatty acid

Fig. 1. Structures of okadaic acid group toxins and pectenotoxins.

in molluscs as well as dinoflagellates such as certain *Dinophysis* and *Prorocentrum* species (Lee et al., 1989). In various mussels and scallop species, 7-*O*-fatty acid esters of these toxins, collectively designated as "DTX3", have also been found. Originally the term "DTX3" referred to a complex mixture of 7-*O*-acyl derivatives of DTX1 (Yasumoto et al., 1985). It has later been demonstrated that also OA and DTX2 can be acylated to give "DTX3" (Marr et al., 1992). High levels of these fatty acid esters of OA, DTX1 and DTX2 have been found in mussels, clams and scallops from Portugal, Spain, Denmark, New Zealand, Japan and Chile (Comesana Losada et al., 1999; Fernandez et al., 1996; Garcia et al., 2004; Jørgensen et al., 2005; MacKenzie et al., 2002; Suzuki and Mitsuya, 2002; Vale and Sampayo, 1999, 2002a,b). The fatty acid derivatives constitute a major part of the total toxin burden in all of these species. The only exception seems to be mussels of the *Mytilus* genera and the clam *Donax trunculus*, where the free forms of OA group toxins generally dominate in the tissue (Fernandez et al., 1996; Jørgensen et al., 2005; Lindegarth et al., in press; Vale and Sampayo, 2002a; Vale, 2006a). The ip toxicity of "DTX3" in mice is reported to be lower than for OA and the *in vitro* biological activity of esters increases with the degree of unsaturation of the fatty acid chain (Yanagi et al., 1989), hence the toxicity of a given sample could depend not only on the amount of toxin present but also on the amount of fatty acid esters in the sample, as well as on the side chains present in the fatty acid moiety.

Pectenotoxins (PTXs) (Fig. 1) are a group of complex cyclic polyether lactone toxins originating from *Dinophysis* species and are commonly found in shellfish around the world. The most commonly found PTX in algae is PTX2, having been identified in *Dinophysis acuta*, *Dinophysis acuminata*, *Dinophysis norvegica*, *Dinophysis fortii* and *Dinophysis rotundata* (Lee et al., 1989; MacKenzie et al., 2002; Miles et al., 2004a; Vale and Sampayo, 2002c), and have also been detected in some *Protoperidinium* species