Available online at www.sciencedirect.com
ScienceDirect
Harmful Algae 6 (2007) 308–316
HARMFUL ALGAE
www.elsevier.com/locate/hal

In vitro transformation of PSP toxins by different shellfish tissues

Elke Jaime [a,*], Gunnar Gerdts [b,**], Bernd Luckas [c]

[a] Friedrich-Schiller-University Jena, Department of Organic Chemistry and Macromolecular Chemistry, Humboldtstrasse 10, 07743 Jena, Germany
[b] Ecological Chemistry, Biologische Anstalt Helgoland, Foundation Alfred Wegener Institute for Polar and Marine Research, Kurpromenade, 27483 Helgoland, Germany
[c] Friedrich-Schiller-University Jena, Department of Food Chemistry, Dornburger Strasse 25, 07743 Jena, Germany

Received 14 November 2005; received in revised form 5 March 2006; accepted 10 April 2006

Abstract

Many in vivo shellfish feeding experiments have been carried out in order to investigate the fate of PSP toxins in the marine food chain. A focal point of these studies concerns the species- and tissue-specific differences in toxin metabolism. However, tissue specific effects are often overlapped by selective toxin retention as well as transfer between individual compartiments. In in vitro experiments presented here digestive tissue and adductor homogenates of 10 shellfish species (bivalvia: *Mytilus edulis*, *Crassostrea gigas*, *Cardium edule*, *Arctica islandica*, *Ensis ensis*, *Modiolus modiolus*, *Mactra stultorum*, *Pecten maximus* as well as two snails: *Littorina littorea* and *Buccinum undatum*) were incubated with an extract of the toxic strain *Alexandrium fundyense* CCMP 1719. After incubation, changes in the toxin pattern could be observed in all samples with significant differences occuring between both the species and tissues. The greatest metabolic activity was found in digestive tissue samples. Among the organisms, the species with a non-filtering lifestyle, *L. littorea* and *B. undatum*, showed the highest conversion rates. Interestingly, the high metabolic transformation rate of the PSP toxins was accompanied with a fast reduction (up to 73%) of toxicity in the homogenates.

Keywords: Bivalvia; Snails; In vitro transformation; Paralytic shellfish toxins

1. Introduction

Paralytic shellfish poisoning (PSP) toxins are potent neurotoxins that are produced by various species of dinoflagellates such as *Alexandrium* spp., *Pyrodinium bahamense* and *Gymnodinium* spp. More than 20 toxin analogues have been reported which differ from one another both in structure and toxicity. PSP toxins can be grouped into three classes in increasing order of toxicity: (1) *N*-sulfocarbamoyl toxins (B1, B2, C1–C4), (2) decarbamoyl toxins (dcGTX1–dcGTX4, dcNeo, dcSTX) and (3) carbamoyl toxins (GTX1–GTX4, Neo, STX). The subsequent flow of these toxins through marine food webs associated with excessive PSP accumulation in shellfish results in harmful impacts on both shellfish fisheries and human health (Botana, 2000). Comparative studies on PSP profiles often show significant differences between the causative dinoflagellates and contaminated bivalves, thus indicating an active toxin metabolism in shellfish (Oshima et al., 1990; Cembella et al., 1994). An understanding of the interactions between shellfish and PSPs is therefore essential for an efficient risk management.

Many controlled in vivo shellfish feeding experiments have been carried out in order to investigate the

* Corresponding author. Tel.: +49 3641 94 82 17; fax: +49 3641 94 82 12.
** Corresponding author. Tel.: +49 4725 81 92 45; fax: +49 4725 81 92 83.
E-mail addresses: elke.jaime@uni-jena.de (E. Jaime), ggerdts@awi-bremerhaven.de (G. Gerdts).

1568-9883/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.hal.2006.04.003

accumulation and fate of PSP toxins in the marine food web. A main focal point of these studies is the species dependency and/or tissue-specific differences in toxin metabolism (see for example: Lassus et al., 1989; Beitler and Liston, 1990; Bricelj et al., 1991; Chen and Chou, 2001; Suzuki et al., 2003). However, tissue-specific effects are often influenced by a variable species intoxification and, in addition, are obscured by toxin transfer between individual tissue compartiments. On the other hand there are only a few investigations reported in the literature that have studied the in vitro transformations of PSP toxins in order to help explain the enzymatic activities of shellfish and the role of bacteria – both of which undoubtedly support the interconversion and degradation of these marine neurotoxins following their accumulation in tissue (Shimizu and Yoshioka, 1981; Kotaki et al., 1985a,b; Oshima, 1995; Murakami et al., 1999; Smith et al., 2001).

The present study reports investigations on the mechanisms involved in the transformation of PSP toxins in extracts of ten common molluscan species of the North Sea. Eight filter feeders were compared to a grazing and a carnivorous snail both of which do not directly take up PSP metabolites from toxic algae. There are remarkably few studies on PSP metabolism of non-filters that are capable of being vectors for toxins in the marine food web (Shumway, 1995; Chen and Chou, 1998; Teegarden et al., 2003). Furthermore, the metabolic capacity of isolated muscle and digestion tissue was compared and species-specific differences were studied as well. These results indicate a widely varying spectrum of toxin conversion activity.

2. Materials and methods

2.1. Shellfish and toxins

The bivalvia *Mytilus edulis*, *Crassostrea gigas*, *Cardium edule*, *Arctica islandica*, *Ensis ensis*, *Modiolus modiolus*, *Mactra stultorum* as well as the herbivore snail *Littorina littorea* and the carnivore snail *Buccinum undatum* were collected by dredging or scuba diving in the German Bay at a site with no historical record of PSP toxicity. *Pecten maximus* was collected by scuba diving off the east coast of Scotland, an area with sporadic records of PSP toxicity. Specimens were kept alive in fresh seawater with non-toxic phytoplankton under laboratory conditions until use. Muscle (MT) and digestion tissue (DT) were dissected from several unisize adult individuals of each species and combined. For *B. undatum*, the digestion tissue was separated into the hepatopancreas (HP) and intestinum (Int) and investigated independently. Only the adductor muscle was examined from *P. maximus*. The tissues were frozen and stored at −80 °C after having been determined to be free of PSP toxins by HPLC (see below).

Algae extract was prepared from *Alexandrium fundyense* CCMP 1719 that had been cultivated in f/2 medium (Guillard, 1975). Cells (23 pg STXeq/cell) were collected by centrifugation and extracted with 0.03N acetic acid (1 min sonication). The extract was analyzed by HPLC (see below) and determined to have a final toxin concentration of 39.940 μg STXeq/ml. It was stored at −20 °C until use.

2.2. Incubation

Aliquots of shellfish tissues (1–2 g) were homogenized (Ultra Turrax) with differing amounts of PBS buffer (pH 6.8) for digestive (1:1, m:m) and muscle (1:2, m:m) tissues depending on their tough consistency. Toxic algae extract (0.2 ml) was added to 1.8 ml of each tissue homogenate. This resulted in toxin concentrations of 34.4 nmol PSP/g for digestive tissue and 51.5 nmol PSP/g for muscle tissue. The mixture was then incubated for 48 h at 16 °C. After this period, the samples were immediately analyzed for PSP toxins without further storing.

Samples were cooled in ice throughout the preparation to prevent enzyme inactivation by heating during homogenization as well as to keep the total enzyme activity at a minimum.

2.3. Toxin analysis

The crude homogenates were centrifuged at 13,000 rpm for 10 min. The retained supernatants were filtered through a 0.22 μm nylon filter and analyzed by HPLC. Algae extract could be directly injected. HPLC was performed by ion-exchange chromatography with fluorescence detection according to the method published by Jaime et al. (2001). A cation-exchange column Source 15S PE 4.6/200 and an anion-exchange column Source 15Q PE 4.6/100 (Pharmacia Biotech) were used to resolve carbamate (GTX, Neo, STX) and decarbamate (dcGTX, dcSTX) toxins. Toxin standards for instrument calibration (STX, Neo, GTX1–GTX4, dcGTX2 and dcGTX3) were purchased from the National Research Council, Marine Analytical Chemistry Standards Program (NRC-PSP-1B), Halifax, Canada. DcSTX was provided by the European Commission (BCR, The Community Bureau of Reference, Brussels). An electrochemical cell (ESA)