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Okadaic acid, a causative toxin of diarrhetic shellfish poisoning, in green-lipped mussels *Perna viridis* from Hong Kong fish culture zones: Method development and monitoring

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Abstract

Green-lipped mussels (*Perna viridis*) were collected from seven fish culture zones (FCZs) in Hong Kong and analyzed for okadaic acid (OA). A conventional HPLC method was modified by incorporating a proteinase K digestion step. Results suggest that a higher recovery (2.5 times higher) of OA was obtained from spiked samples after the addition of 1.08 mg proteinase K in comparison with samples incubated without the proteolytic enzyme. For the hepatopancreas (HP) of individual field-collected mussels, the additional digestion step can enhance OA extraction by 3.1 times. Spatial and temporal variations in OA concentrations in the mussels from various FCZs were investigated. The highest concentration of OA in mussel HP samples was 1164.9 ng/g HP wet wt. With respect to OA concentrations in whole mussel tissues from seven sites and four seasonal samplings, the concentrations were between 70.0 and 131.0 ng/g wet wt., which did not exceed the generally recognized international regulatory criteria (>200 ng/g) for OA.

Keywords: Diarrhetic shellfish poisoning; Okadaic acid; *Perna viridis*; Fish culture zone; Hong Kong

1. Introduction

Diarrhetic shellfish poisoning (DSP) is a gastrointestinal illness with symptoms such as diarrhea, nausea, vomiting, headache, chills and moderate to severe abdominal pain (Quilliam and Wright, 1995). The first reported cases of DSP were in the Netherlands in the 1960s, followed by similar reports in the late 1970s from Japan (Aune and Yndstad, 1993). Since then, numerous cases have been reported from many parts of the world, including Europe, South America, North America, Asia, Australia and New Zealand (Yasumoto et al., 1980; Aune and Yndstad, 1993; Quilliam and Wright, 1995). Indeed, the occurrence of DSP is of worldwide public health concern, and poses a hazard to the shellfish industry in many countries particularly in Europe and Asia (Yasumoto and Murata, 1993).

DSP is usually a consequence of consuming contaminated shellfish that have ingested large quantities of toxic dinoflagellates of the genera *Dinophysis* and *Prorocentrum* through their filter feeding activities (Yasumoto et al., 1985; Lee et al., 1989). Specifically, the main causative organisms, *Dinophysis* dinoflagellates, are known to produce at least nine toxins (Yasumoto et al.,

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1980). Okadaic acid (OA), one of the principal causative toxins of DSP, is a complex, high molecular weight, lipophilic polyether.

OA was first isolated from the sponge *Halichondria okadai*, and has been known to stimulate the phosphorylation of proteins that control sodium secretion by intestinal cells (Cohen et al., 1990). OA may also enhance the phosphorylation of cytoskeletal or junctional elements which regulate permeability to solutes, thereby resulting in diarrheagenic effects of passive loss of fluids (Dho et al., 1990). OA is amongst the first recorded compounds which inhibit protein phosphatases 1 (PP1) and 2A (PP2A). These phosphatases are two of the four major enzymes that dephosphorylate serine/threonine residues of proteins in eukaryotic cells, and an important group of enzymes that are involved in many crucial cellular metabolic processes (Cohen, 1989). Bialojan and Takai (1988) pointed out that OA and related compounds could bind to receptor sites on PP1 and PP2A and inhibit their enzymatic activities in dephosphorylation. Indeed, OA has been described as a powerful tumour promoter due to its inhibitory effects on protein phosphatases (Suganuma et al., 1988; Haystead et al., 1989; Fujiki and Suganuma, 1993).

Based on the mouse assay method of the Japanese Ministry of Health and Welfare (1981), 4.0 μg of OA would correspond to one mouse unit (MU), defined as the amount of toxin that kills a mouse in 24 h. The regulatory level in several countries has been set at 0.05 MU/g whole tissue. The regulatory level of OA for safe human consumption should therefore be equivalent to 200 ng/g whole tissue. These are the currently recognized actions levels for OA in the US and Australia. However, because of differences in sampling and detection techniques, the action levels of diarrhetic shellfish toxins (DSTs) can vary significantly between other countries. There is no regulatory limit for DSTs in the European Union (EU). The relevant EU legislation states that there should be no positive result by mouse bioassay. There are no numerical regulatory action concentrations for Hong Kong.

Although the mouse bioassay has been used for the determination of DSTs for a long time, this bioassay method has a number of drawbacks. It is time-consuming, and has low reproducibility. There is also the ethical issue involving the use of animals in testing. Furthermore, the bioassay is susceptible to the presence of contaminants irrelevant to DSP. For instance, free fatty acids, which can rise to high levels in shellfish digestive glands during spring and early summer, often cause false positive results (Lee et al., 1987). There is a need for alternative methods that are rapid, sensitive, specific and reproducible for detecting OA. One of the alternatives available to date is the high performance liquid chromatograph (HPLC) method, which has been used successfully for verification of false positives and for removing the interpretative doubts that may arise with a mouse bioassay.

To date, the HPLC method of Lee et al. (1987) is one of the most commonly used analytical techniques for the determination of OA and its derivatives in mussels. In this paper, we report upon a modification of this conventional HPLC method with an incorporation of a proteolytic digestion step by proteinase K. Proteinase K is a non-specific serine protease, which has a very high specific activity. It is commonly used to purify target material from contaminating proteins by proteolysis. After validation of the modified procedure, it was used to measure OA concentrations in mussel (*Perna viridis*) samples collected from seven fish culture zones (FCZs) in Hong Kong. This investigation provided the first baseline data on OA concentrations in marine shellfish from the South China region.

2. Materials and methods

2.1. Mussel sampling

Mussels were obtained from a monitoring programme for the analysis of OA undertaken in conjunction with the Agriculture, Fisheries and Conservation Department (AFCD) of the Hong Kong Government. In this monitoring programme, 30 specimens of the green-lipped mussel, *Perna viridis*, (shell length: 7–10 cm) were collected from each of seven fish culture zones (FCZs), namely Kat O (KO), O Pui Tong (OPT), Tap Mun (TM), Kau Sai (KS), Lo Tik Wan (LTW), Man Wan (MW) and Yim Tin Tsai (YTT) (Fig. 1), between January 2002 and April 2003.

2.2. Modification of HPLC method

To investigate any matrix effects due to the mussel tissues in OA analysis, the hepatopancreas (HP) (known to contain over 98% of OA in mussels (Edebo et al., 1988)), was dissected from 50 mussels collected randomly from the FCZs, pooled and then completely homogenized using an Ultra-Turrax T8 homogenizer. The background OA level in the pooled HP sample was determined by HPLC with fluorescence detection (Quilliam and Wright, 1995), and the background data were used to correct for OA concentrations determined in OA-spiked HP samples in subsequent analyses. Homogenized HP samples were spiked, in triplicate, with 0, 150, 300, 600, 1200, 2400, 4800 ng OA, and incubated at 4 °C for 24 h. The OA concentrations in HP tissue samples were then determined by HPLC, with fluorescence detection. The results obtained from HP tissues were compared with OA concentrations determined in methanol spiked with the same amounts of OA standards (Calbiochem, La Jolla, CA) as above.