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Distribution and linkage of domoic acid (amnesic shellfish poisoning toxins) in subcellular fractions of the digestive gland of the scallop *Pecten maximus*

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ABSTRACT

The king scallop *Pecten maximus* accumulates domoic acid, the main amnesic shellfish poisoning toxin, in the digestive gland for a long time. To try to find if the cause of this characteristic is the binding of the toxin to some cellular component, the subcellular distribution of domoic acid in the cells of the digestive gland was studied, by means of serial centrifugation, ultrafiltration and size exclusion chromatography (SEC). Domoic acid was found to be present mostly in soluble form in the cytosol, as more than 90% was found in the supernatant after a centrifugation of 1 h at 45,000 × *g*, and passed a 10 kDa ultrafilter. The retention time of the peak with an absorption maximum of 242 nm – the one characteristic of domoic acid – observed in the SEC chromatograms of the scallop samples was found identical to be one of a reference solution of the toxin, indicating therefore, that domoic acid is free in the cytosol of the digestive gland of *Pecten maximus*. This finding turns the focus from binding to the lack of membrane transporters in this species of the scallop as the cause of the long retention time of domoic acid in this species.

1. Introduction

Since the outbreak of the amnesic shellfish poisoning (ASP) in 1987 in Canada, produced by the accumulation of domoic acid in bivalves (Bates et al., 1988; Wright et al., 1989), numerous events of this type of toxicity have been detected throughout the world. Exploitable populations of molluscs (Gilgan et al., 1990; Haya et al., 1991; Míguez et al., 1996; James et al., 2005; Wekell et al., 1994a, b; Vale and Sampayo, 2001; Campbell et al., 2001; Costa et al., 2004), crustaceans (Lund et al., 1997; Wekell et al., 1994a; Ferdin et al., 2002) and fishes (Costa and Garrido, 2004a; Busse et al., 2006; Lefebvre et al., 2002; Wekell et al., 1994a) have been affected by this kind of toxicity that consequently has become increasingly important both, ecologically and economically.

In bivalves, the depuration rate of these toxins is species-specific and highly variable. Some species depurate domoic acid quickly, as the mussels *Mytilus edulis* (Gilgan et al., 1990; Wohlgeschaffen et al., 1992; Novaczek et al., 1992), *Mytilus californianus* (Whyte et al., 1995), *Mytilus galloprovincialis* (Blanco et al., 2002) or the soft shelled clam *Mya arenaria* (Gilgan et al., 1990), while other accumulate it for a long time, such as the horse mussel *Volsella modiolus* (Gilgan et al., 1990), the sea scallop *Placopecten magellanicus* (Gilgan et al., 1990; Douglas et al., 1997) and specially the king scallop *Pecten maximus* (Blanco et al., 2002), and the razor clam *Siliqua patula* (Drum et al., 1993; Horner et al., 1993; Wekell et al., 1994a, b; Adams et al., 2000; Trainer and Bill, 2004).

In Galicia, and very likely in other locations, the extremely slow depuration in *Pecten maximus* (0.007 day^{-1}, Blanco et al., 2002), together with the recurrent appearance of outbreaks

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of this kind of toxicity, caused by algal blooms which usually occur following a seasonal pattern (Pazos et al., 2003; Salgado et al., 2003; Moroño et al., 2004) cause, in some cases, banning periods of several years due to the reintoxication of the scallops with new *Pseudo-nitzschia* blooms before being able of completely depurating the toxins accumulated from the previous bloom. Even though this situation became less dramatic after the Decision of the European Union 2002/226/EC – which allows harvesting and marketing selectively eviscerated *Pecten maximus* and *Pecten jacobeus* under special conditions – the problem is still important.

In order to mitigate the impact of these outbreaks, it is necessary to find a method to accelerate depuration to, at least, avoid the coupling of the toxin accumulation in consecutive phytoplankton blooms. Blanco et al. (2006) found that relocation of natural bed scallops in rafts accelerates considerably the depuration process, but the actual causes of the acceleration are not known, making it difficult to implement additional modifications to improve the obtained response.

As the slow depuration in *Pecten maximus* is caused by the low depuration rate of domoic acid from the digestive gland (Blanco et al., 2002, 2006), usually, more than 90% of the domoic acid is accumulated in this organ (Arévalo et al., 1998). We have hypothesized that this high sequestering capability should be due to intracellular compartmentalisation or to the binding of the toxin to some cellular receptors as it was found in *Siliqua patula* – the other species with extremely low depuration rate – by Trainer and Bill (2004). In order to check this hypothesis, in this work, we have studied the subcellular distribution and the possible binding of domoic acid in the digestive gland of scallop *Pecten maximus*. The study was carried out by means of the analysis of the subcellular fractions obtained by centrifugation, ultrafiltration and size exclusion chromatography.

2. Materials and methods

2.1. Biological material

The scallops were collected from the Ría de Arousa (Galicia, NW Spain) after an ASP outbreak and held in a tank with running seawater until the experiment was carried out. As digestive gland is the target organ for these toxins, in this work, only digestive glands were studied.

2.2. Dissection and homogenisation

In order to avoid the possible release of the bound toxin by cleavage of its binding with the receptor, if there was any, three treatments were applied to the samples previously or during homogenisation: (1) thermal deactivation on the enzymes (Experiment 1); (2) inhibition of the protease activity in the samples (Experiment 2); and (3) inhibition of the activity associated to thiol groups (Experiment 3).

Three digestive glands were used in each experiment. Glands were carefully dissected, weighed and homogenised in a buffer solution (5 mL of buffer/1 g of digestive gland) with an Ultraturrax (3 min, 12,000 rpm) while refrigerated by maintaining them in an ice bath. The buffer solution was made of 0.1 M Tris pH 7.8 and ammonium formate 0.15 M (base buffer) with some complements in the second and third experiments.

In Experiment 1 only the base buffer was used, but the enzymatic activity was suppressed by autoclaving the digestive glands (110 °C, 5 min) previously to the homogenisation. In Experiment 2, the base buffer was complemented with a protease inhibitor cocktail P2714 from Sigma–Aldrich. One bottle of P2714 was dissolved in buffer following the procedures suggested by the manufacturer, giving the following final concentrations of the cocktail

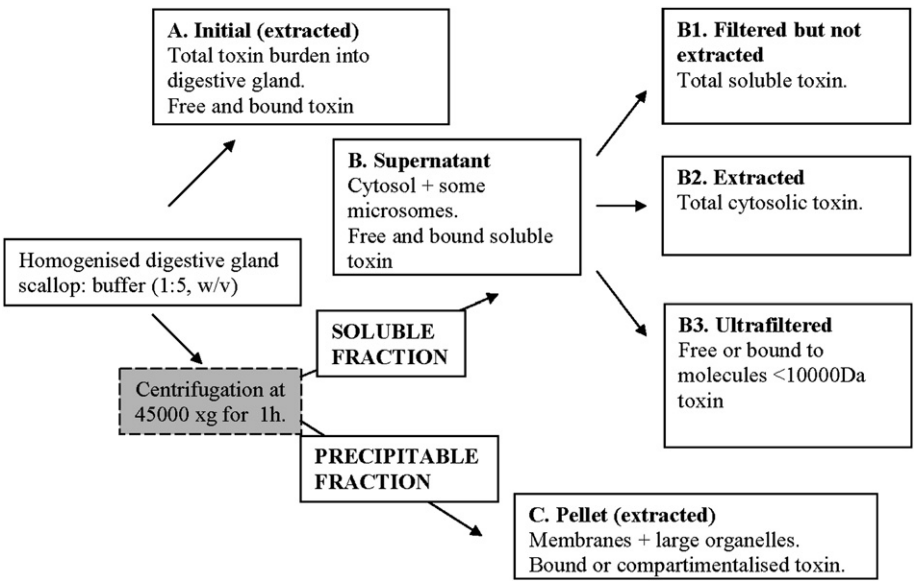

Fig. 1. Fractionation scheme with its corresponding subcellular components and the forms of the toxin in each of them.