Paralytic Shellfish Toxins: a complex group in constant (bio)transformation

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Abstract

Paralytic shellfish toxins (PSTs) are a large group of marine biotoxins (~50 analogues), mainly produced by marine dinoflagellates of the genera *Alexandrium*, *Gymnodinium* and *Pyrodinium*, which are more typical in tropical and temperate climate zones. All members of the toxin group share a common core structure. Still, the combination of different chemical functionalities defines the subgroup of each analogue (e.g., carbamoyl, *N*-sulfocarbamoyl, decarbamoyl, benzoyl), influencing its toxicological action and determining the level of toxicity of each member. PSTs are prone to biotransformations within living organisms, affording analogues with higher or lower toxicity. Such biotransformations may be mediated by different agents (e.g., enzymes, natural reducing agents, bacteria) in different living organisms undergoing chemical processes, leading to diverse outcomes. This work intends to highlight the main reactions (bioconversions) that occur in living organisms (dinoflagellates, bivalves, and humans) and relate changes in molecular structure, caused by such responses, to toxicity. Additionally, we also present research under development aiming to create chemical solutions for the decontamination of bivalve molluscs, thus helping to minimize this problem's social and economic impacts.

Keywords: marine biotoxins, saxitoxins, bioconversion, humans, bivalves, dinoflagellates

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Introduction

Harmful algae blooms (HABs) are natural phenomena characterized by the growth of phytoplankton in marine ecosystems, producing highly potent natural toxins, called phycotoxins or marine biotoxins (Egmond, 2004), that cause great social concern and economic losses worldwide. These toxins bioaccumulate in aquatic species, such as bivalves, and may be potentially toxic for humans (through the food chain). Among HABs episodes, paralytic shellfish toxins (PSTs) stand out by their relatively large contribution to the total. Recently published data referring to the period between 1985 and 2018 revealed that the highest percentage (35 %) of the events associated with seafood toxins was attributed to PSTs (Hallegraeff *et al.*, 2021 a, b).

PSTs, also recognized as saxitoxin (STX) and its analogues, or simply saxitoxins (STXs), are a large and diverse group of marine biotoxins responsible for paralytic shellfish poisonings (PSP) (Wiese *et al.*, 2010; Leal and Cristiano, 2022). This group of toxins is of particular concern for its effects on human health, as ingestion of high amounts may lead to death within a few hours. According to a dose-response modelling of PSTs in humans, the lower critical dose with a probability higher than 10% of death is 82.2 µg STX eq kg^{-1} b.w. (Arnich and Thébault, 2018). An antidote or therapy is not known and only some clinical measures are possible at an early stage of intoxication (Montebruno, 1993; Belin and Raffin, 1998; FAO, 2004). 4-aminopyridine seems to reverse sublethal effects of saxitoxin, however no studies in humans have yet been performed (Chang *et al.*, 1997; Nguyen *et al.*, 2021).

Structurally, saxitoxins share a common core, depicted in figure 1, comprising a 3,4-propinoperhydropurine tricyclic system and two guanidine groups, accounting for the hydrophilic and highly polar character of these molecules. The PSTs sub-groups are defined according to their characteristics, based on the substituent group in R4. Most toxins belong to one of the subgroups shown in figure 1, namely the carbamoyl, *N*-sulfocarbamoyl, benzoyl, and decarbamoyl subgroups. Each substituent group affects the binding affinity to the receptor, by steric hindrance, net charge and/or polarity (Leal and Cristiano, 2022). Within each subgroup, the molecules may or may not suffer hydroxylation (R1) at N1, making possible the distinction between hydroxylated and non-hydroxylated toxins. Also, R2 and/or R3 may be replaced by -H, $-OSO_3^-$ or -OH groups, originating numerous possible combinations. Furthermore, the orientation of the groups at C11 (R2 and R3) defines the stereochemistry of the molecules and may influence their binding affinity to the receptor.

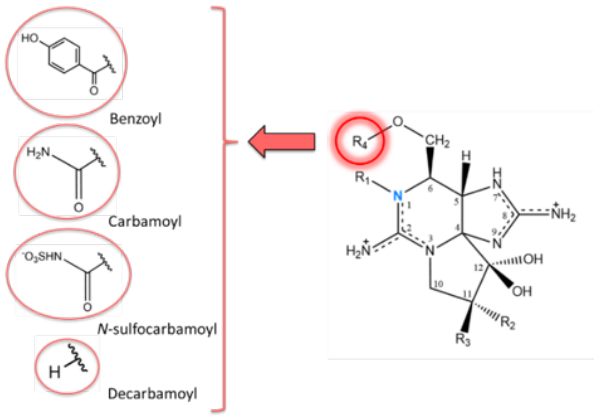

Fig. 1. Core structure of PSTs and identification of the main substituent groups in R4.

Our aim in this study was to highlight the different bioconversion reactions that occur among this group of toxins, discussing the organisms and the conditions in which they predominantly occur, as well as the impacts that those transformations have on their level of toxicity. We also describe our progress in mitigating the socio-economic effects caused by the accumulation of biotoxins in bivalves.

Bioconversions in organisms

The complexity of this group of toxins is due not only to their high number of analogues, but also to their ability to interconvert within organisms, namely, dinoflagellates, bivalves, and humans. There are some common bioconversion reactions to the different organisms. The main ones, compiled in figure 2, are grouped according to the organism where they were observed. Oxidation at N1 is a process common to bivalves, humans, and dinoflagellates. The introduction of a sulfate group (sulfonylation) has been observed in humans and dinoflagellates and may occur in R4 (at N-21) or R2/R3 (at O-22, the oxygen bound to C11) substituent groups. In turn, hydroxylation has been reported to occur in bivalves and dinoflagellates, while reductive cleavage of the *O*-sulfate group has been observed in bivalves and humans. Both reactions were documented to take place at C11. Lastly, hydrolysis of the carbamoyl ester (in R4 substituent group), originating the correspondent decarbamoyl analogues, is frequently reported in bivalves, and in humans. Hydrolysis of the *N*-sulfa group (side chain, in R4) has also been reported in bivalves. In addition to all these reactions, there are others that seem to be more characteristic of a specific organism. For example, as far as we currently know, the reductive elimination of the *N*-hydroxyl group (at N1), as well as the *O*-desulfonylation (at C11) are reactions only described in bivalves, while in humans a very specific reaction has been proposed: glucuronidation (García *et al.*, 2009, 2010). This reaction occurs at hydroxyl C12 and assumes an important role in accelerating the detoxification process, as it produces more hydrophilic molecules, which favours the excretion of toxins by the body. Most of these reactions in organisms are mediated by enzymes (e.g., carbamoylases, sulfo- or glucuronosyl-transferases, oxidases), but the involvement of natural reducing agents, like glutathione (Sakamoto *et al.*, 2000), and bacteria (Smith *et al.*, 2001) has been reported in some metabolic transformations, namely reductive cleavage (glutathione and bacteria) and reductive elimination (bacterial isolates).

All these reactions may occur naturally and/or may be potentiated by several factors, such as pH, temperature, and even by sample handling procedures prior to analysis (Vale, 2008).

(Bio)conversions may change the toxicological profile in each organism and the overall toxicity. It is difficult to establish "rules" because the same type of reaction may not induce the same consequence in terms of toxicity, mainly if different sub-groups of toxins (based on the R4 classification) are considered. This is due to the introduction of substituent groups that cause a change of the molecular structure, also implying alterations of the physicochemical properties (e.g., pKa, net charge, polarity and/or steric hindrance) of the molecules, which leads to greater or lesser binding affinity with the receptors and, consequently, greater or lesser toxicity.

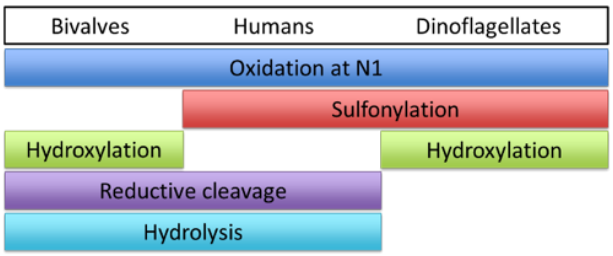

Fig. 2. Common bioconversion reactions reported in bivalves, humans, and dinoflagellates.

Considering the most recent proposal by international entities (FAO/WHO, 2016), based on the toxicity equivalency factor (TEF), the carbamoyl sub-group is the most toxic (2 ≥ TEF ≥ 0.4), followed by decarbamoyl (0.5 ≥ TEF ≥ 0.2) and *N*-sulfocarbamoyl (0.1 ≥ TEF ≥ 0.01) sub-groups. The TEF for toxins belonging to benzoate sub-group are not yet established, but some studies showed these analogues are only slightly less potent than saxitoxin (Llewellyn *et al.*, 2004), whose TEF is equal to one. More recent studies, using a docking simulation approach, seem to corroborate the toxin potency of these analogues (Durán-Riveroll *et al.*, 2016). These authors found some benzoyl analogues bound strongly to the $Na_v1.4$ channel.

Considering the above relation between the sub-groups and their toxicity, hydrolysis seems to be the most impactful reaction in the toxicity of these molecules. On the one hand, hydrolysis of the carbamoyl ester, converting carbamoyl/*N*-sulfocarbamoyl/benzoyl toxins into decarbamoyl analogues (e.g., STX to dcSTX), decreases the toxicity of the molecule (Leal and Cristiano, 2022). On the other hand, hydrolysis of the *N*-sulfa group (side chain, in R4), converting *N*-sulfocarbamoyl toxins into the correspondent carbamoyl, increases the toxicity of the molecule (e.g., gonyautoxin 5 (GTX5) to STX). Also, sulfonylation at N-21 (side chain, R4) impacts on the toxicity of the molecules. The introduction of a sulfate group at this site, originating *N*-sulfocarbamoyl toxins from carbamoyl toxins (e.g., STX to GTX5), seems to promote a decrease in toxicity. A more detailed and in-depth discussion about this topic may be found in our recent review article (Leal and Cristiano, 2022).

Ongoing work

The presence of marine biotoxins in bivalve molluscs represents a worldwide problem and leads to great economic losses, resulting from the prohibition of their harvesting. For example, data from 2018 indicate that, in southern Portugal (Algarve), the restrictions on harvesting of mussel, cockle and clam due to the presence of marine biotoxins reached an average of 124, 88, and 42 days, respectively (IPMA, 2018). To secure the economic and human health of HAB affected regions, it is critical to develop effective and safe strategies that contribute to solving this problem. The goal of our project, "DEPURATOX", MAR-01.03.01-FEAMP-0049 (2020-2022) is the development of a product that is capable of decontaminating, *in vivo*, bivalves containing biotoxin levels above the legal limits, in order to avoid the devaluation of this food, either by the death of the bivalves and the consequent need for processing to sale, or by the complete ban on their harvest. The structure and chemical nature of the solutions under development take advantage of the molecular properties and physicochemical characteristics of the toxins, whose structures are known, informing the design to enhance the effectiveness of interventions. Studies have been performed with PSTs, but future studies

with diarrhoeic shellfish toxins (DSTs) are also planned. Updates related to this project may be found on the website (https://www.researchgate.net/project/DEPURATOX).

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