BIOCHEMISTRY

Enzymes that detoxify marine toxins

Potent microbial toxins found in shellfish are possible starting points for drug discovery, but analogues are needed for biological testing. Toxin–modification enzymes now suggest a new approach for producing these analogues.

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There is an acute need for new medications to treat pain. Important sources of therapeutics for pain management and other human conditions are natural products — complex, biologically active small molecules made by living organisms. But compounds isolated directly from natural sources often do not have the optimal properties to be drugs. Therefore, a major challenge faced by those using natural products as leads for drug discovery is how to access a diverse range of closely related molecular structures for biological testing. This can be accomplished using chemical synthesis, but the complex structures of natural products often make that approach challenging.

The difficulties of accessing structural analogues have hampered efforts to investigate a family of natural products called paralytic shellfish toxins (PSTs) as candidate therapeutics for pain[1]. Many PSTs are highly potent (they elicit a strong response from their molecular biological targets) and are therefore highly toxic, which has hindered their development as drugs and has generated interest in accessing less potent analogues. Writing in *ACS Chemical Biology*, Lukowski *et al.*[2] report the biosynthetic pathway that generates PSTs to which sulfo groups (SO_3^-) have been added, which are less toxic members of this family of compounds. The sulfotransferase enzymes characterized in the study modify extremely complex substrate molecules, and therefore might facilitate access to other less toxic analogues of PSTs for drug development.

PSTs are produced by marine microorganisms, including cyanobacteria and dinoflagellates[3,4]. They are responsible for the numbness, tingling and more-severe symptoms of paralytic shellfish poisoning (caused by eating shellfish contaminated with these toxins), and interfere with the voltage-gated sodium channels that are responsible for transmitting signals in the nervous system. Previous efforts to isolate PSTs revealed that microbes often make analogues that bear one or more sulfo groups, leading to the discovery that this chemical modification reduces the potency and toxicity of these natural products[5,6].

The biosynthetic pathways and enzymes involved in the installation of these sulfo groups were not understood until a few years ago. The first insights were obtained from assays that used poorly characterized enzyme preparations isolated from dinoflagellates[7,8]. These studies suggested that the sulfo groups were probably added to PSTs at a late stage of the biosynthetic pathway. More recently, the identification of the cyanobacterial genes encoding the biosynthetic machinery that produces saxitoxin, a highly potent PST, have enabled a molecular understanding of PST assembly[9].

Saxitoxin is assembled through transformations that convert the amino acid L-arginine into a series of increasingly elaborate structures. Previous work[10] had identified two putative sulfotransferase enzymes (SxtN and SxtSUL) encoded by saxitoxin's biosynthetic gene clusters, and had found that SxtN can attach a sulfo group to a particular nitrogen atom in saxitoxin to generate an analogue called gonyautoxin 5 (Fig. 1). However, the position on saxitoxin at which the second sulfotransferase (SxtSUL) installs a sulfate (SO_4^-), and the order in which the enzymes are used in nature, were not determined.

Lukowski *et al.* have now characterized the ability of purified SxtN and SxtSUL to modify saxitoxin and other PSTs. Researchers from the same group had previously shown[11] that an oxygenase enzyme called GxtA catalyses the selective addition of a hydroxyl (OH) group to a normally unreactive carbon centre in saxitoxin (Fig. 1). In the current work, the authors combined SxtN and SxtSUL with GxtA, and thereby not only confirmed that SxtN installs a sulfo group on the previously identified nitrogen atom, but also discovered that SxtSUL selectively sulfates the hydroxyl group generated by GxtA.

Unlike the non-enzymatic transformations typically used in the chemical synthesis of natural products, these enzymatic reactions are highly selective for single sites on the PST scaffold, and tolerate the presence of the many densely packed, reactive chemical groups that are embedded in the complex molecular architecture of PSTs. Lukowski *et al.* were therefore able to produce a variety of sulfated PSTs directly from saxitoxin. When they measured the biological activity

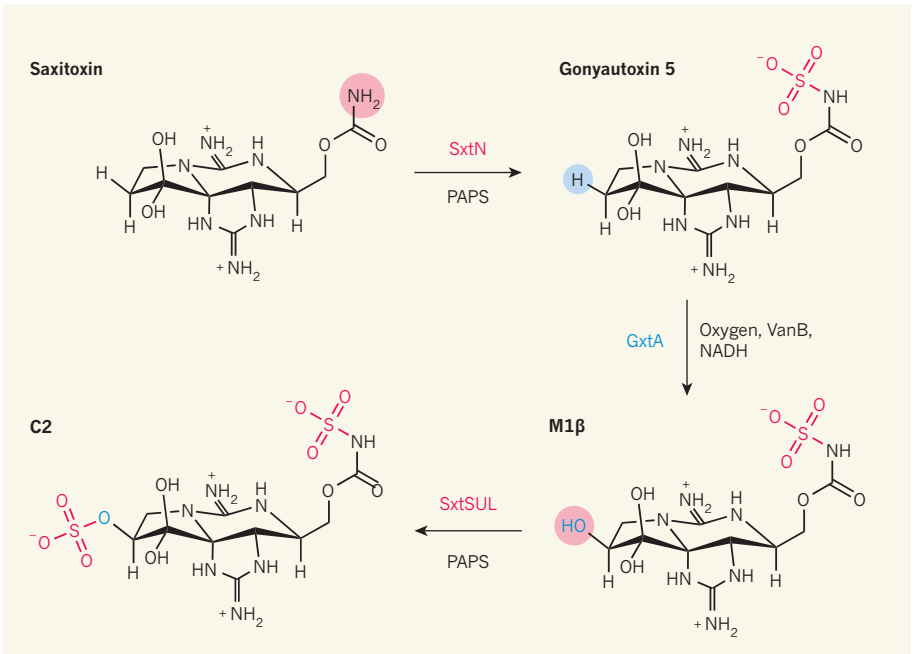

Figure 1 | Biosynthesis of sulfated paralytic shellfish toxins (PSTs). PSTs, including saxitoxin, are potentially fatal to humans, but less toxic analogues are potential leads in the search for new painkillers. Lukowski *et al.*[2] have worked out the biosynthetic pathway that converts saxitoxin into less toxic sulfated analogues in microbes. They find that SxtN, a sulfotransferase enzyme, attaches a sulfo group (SO_3^-) specifically to a nitrogen atom in saxitoxin, forming the compound gonyautoxin 5. The GxtA enzyme then selectively adds a hydroxyl (OH) group to the other end of the molecule, forming M1β, and a second sulfotransferase, SxtSUL, converts the hydroxyl group into a sulfate group (SO_4^-), forming the C2 analogue. The work might allow less toxic sulfated PST analogues to be prepared using a combination of conventional chemical synthesis and enzymatic chemistry. PAPS and NADH are enzyme cofactors; VanB is a partner enzyme of GxtA.

of these compounds, the results confirmed that the addition of multiple sulfo groups to PSTs reduces the compounds' binding affinities to voltage-gated sodium channels. This strongly suggests that sulfo groups reduce PST toxicity, further highlighting their potential for incorporation into PST-based drug candidates.

The use of biosynthetic enzymes to modify PSTs represents a strategy that is distinct from the chemical-synthesis approaches more frequently used to make analogues of these natural products[12]. Although many of those synthetic efforts have been successful, they often involve long sequences of reactions and deliver low yields of products as a consequence of the challenging architectures of the PSTs — which contain an abundance of reactive oxygen and nitrogen atoms that complicate the use of more-standard chemical reactions. Lukowski and colleagues' findings now offer researchers the opportunity to combine conventional synthetic chemistry with biocatalysis, using enzymes to further modify PST scaffolds obtained by synthetic routes. This could potentially streamline access to sulfated versions of these natural products. It might eventually even be possible to use this approach to make non-natural PST analogues for evaluation as candidate therapeutics.

However, substantial barriers must be surmounted before these sulfotransferase enzymes can be fully integrated into PST syntheses. Their catalytic efficiency is very low, and they have not yet been used on a large scale — Lukowski and colleagues worked at a sub-milligram scale, but multi-gram quantities of PST analogues would eventually be needed for the preclinical development of drug candidates. Also, the reactivity of the enzymes towards non-natural PST scaffolds, or towards members of related toxin families, has yet to be evaluated. If the reactivity and selectivity of the sulfotransferases can be optimized using enzyme engineering, these biocatalysts will become powerful synthetic tools in the search for new pain therapeutics. ■

"These findings offer researchers the opportunity to combine conventional synthetic chemistry with biocatalysis."

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1. Durán-Riveroll, L. M. & Cembella, A. D. *Mar. Drugs* **15**, 303 (2017).
2. Lukowski, A. L. *et al. ACS Chem. Biol.* **14**, 941–948 (2019).
3. van Apeldoorn, M. E., van Egmond, H. P., Speijers, G. J. A. & Bakker, G. J. I. *Mol. Nutr. Food Res.* **51**, 7–60 (2007).
4. Bustillos-Guzmán, J. J. *et al. Food Addit. Contam. A* **32**, 381–394 (2015).
5. Andrinolo, D., Michea, L. F. & Lagos, N. *Toxicon* **37**, 447–464 (1999).
6. Andrinolo, D., Iglesias, V., Garcia, C. & Lagos, N. *Toxicon* **40**, 699–709 (2002).
7. Sako, Y. *et al. J. Phycol.* **37**, 1044–1051 (2001).
8. Yoshida, T. *et al. Fish. Sci.* **68**, 634–642 (2002).
9. Wang, D.-Z., Zhang, S.-F., Zhang, Y. & Lin, L. *J. Proteom.* **135**, 132–140 (2016).
10. Cullen, A. *et al. ACS Chem. Biol.* **13**, 3107–3114 (2018).
11. Lukowski, A. L. *et al. J. Am. Chem. Soc.* **140**, 11863–11869 (2018).
12. Berlinck, R. G. S., Bertonha, A. F., Takaki, M. & Rodriguez, J. P. G. *Nat. Prod. Rep.* **34**, 1264–1301 (2017).

This article was published online on 10 June 2019.

IMMUNOLOGY

A licence to kill during inflammation

Inflammasomes are protein complexes that fight infection by driving inflammation or cell death. It now seems that the protein NEK7 provides a 'licence' for the formation of inflammasomes containing the protein NLRP3. SEE ARTICLE P.338

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Inflammation can help to eliminate infection, but excessive inflammation can cause damage to the body. The sensor proteins that trigger an inflammatory immune response must therefore be carefully regulated. Some intracellular immune-sensor proteins detect components in a cell that become abnormal or altered during a cellular crisis. Signs of cellular crisis are sometimes produced in the absence of an infection, so mechanisms are needed to prevent the proteins from triggering an inappropriate inflammatory response. Sharif *et al.*[1] report a structural study on page 338 that investigates an immune-sensor protein called NLRP3, revealing that a protein called NEK7 acts as a 'licence' that enables this protein to cause inflammation.

When an immune sensor recognizes a hallmark of infection in the cytoplasm, this can activate the protein and lead to the assembly of a multiprotein complex called an inflammasome. The activation of proteins that function downstream of an inflammasome can potently drive both inflammation and cell death[2]. Different types of inflammasome can form depending on the sensor components involved. Certain inflammasomes respond to a highly specific trigger: for example, those in mammalian cells containing the sensor protein NLRC4 respond to the presence of the bacterial protein flagellin[3,4].

Proteins that are normally present in mammalian cells do not seem able to trigger the accidental formation of NLRC4-containing inflammasomes, given the lack of reports of such aberrant events. By contrast, inflammasomes that contain NLRP3 are activated when NLRP3 recognizes — by an as yet unknown mechanism — hallmarks of cellular catastrophe, such as extremely low concentrations of potassium in the cytoplasm, or signs of dysfunction in organelles called mitochondria[2]. Such events can arise from tissue damage that is unrelated to infection, and NLRP3 activation in such cases has been implicated as a possible cause of inflammatory diseases such as atherosclerosis.

It is widely accepted that the tightly regulated formation of NLRP3-containing inflammasomes occurs in two steps. In the first step, NLRP3 is primed for action by other immune-sensor proteins called TLRs, which can detect components of microorganisms. This priming step occurs in two ways[2]: NLRP3 can undergo a modification, such as the addition of a phosphate group or the removal of an attached ubiquitin protein. Further priming is achieved by a rise in expression of the gene that encodes NLRP3, increasing the chance that NLRP3 will detect any abnormalities. The second step, activation, then results in NLRP3 proteins binding together to form part of a disc-shaped inflammasome complex that is probably similar to those of other inflammasomes containing proteins of the NLR family (which includes NLRP3 and NLRC4)[5,6]. This activation step occurs during a cellular catastrophe, but the biochemical and structural mechanisms involved are unknown.

Researchers have long sought to determine the structure of NLRP3 as it forms an inflammasome, in the hope of gaining insights into how this protein functions. However, such efforts have been unsuccessful, perhaps because unknown protein partners that interact with NLRP3 were missing from earlier attempts.

The discovery[7–9] that the enzyme NEK7 is essential for NLRP3 signalling provided a missing part of the puzzle. NEK7 regulates processes that occur during cell division, such as the breakdown of the nuclear-envelope structure[10], so it was surprising to find that it has a separate role in inflammation. This suggested that NLRP3-containing inflammasome formation doesn't occur during cell division because NEK7