Enzymes that detoxify marine toxins

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. 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, Łukowski et al. 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 sulftoferase 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. 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.
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. 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. Łukowski 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 sulfolystransferase 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 — Łukowski 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 sulfolystransferases 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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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: 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)8,9. 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 of 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 structure30, 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

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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. 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. 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.4–6 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 — hallmark properties of cellular catastrophe, such as extremely low concentrations of potassium in the cytoplasm, or signs of dysfunction in organelles called mitochondria. 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.