The substrate lends a hand

Duramycin is a small post-translationally modified peptide with antibody-like affinity for phosphatidylethanolamine. As it turns out, the same functionality that is essential for duramycin activity helps to catalyze the formation of its conformationally constrained and compact polycyclic architecture.

Albert A. Bowers

Post-translational modifications (PTMs) on proteins and peptides play important roles in genome structure and regulation. PTMs are also used in nature to synthesize bioactive molecules related to bacterial signaling and defense. In particular, much sophisticated enzymatic chemistry is used in the biosynthesis of ribosomally encoded and post-translationally modified peptides (RiPPs), a broad class of peptide-based natural products[1]. RiPP biosynthetic logic favors the evolution of complex peptide architectures with exquisite near-antibody-like affinity and selectivity for their particular targets. Studies have shown that the PTM enzymes involved in RiPP biosynthesis are relatively promiscuous, thus allowing for variations in the peptide sequence to be tested over the course of the evolution and natural selection of final compounds with optimal biological activity. These enzymes ultimately install a broad array of chemical modifications with high degrees of site-, regio-, and stereoselectivity. Now, characterization of DurN unveils a novel biochemical strategy for ensuring the selection of key active motifs in the RiPP natural product duramycin[2].

Duramycin, a member of the class of RiPPs known as lantipeptides, exhibits valuable pharmacological activity. Importantly, duramycin promotes transbilayer phospholipid movement and binds phosphatidylethanolamine (PE) in the cell membrane of mammalian cells[3]. This activity has made duramycin an essential tool for researchers studying PE flux and has also led to its entry into phase II clinical trials for treatment of cystic fibrosis. Additionally, duramycin is being considered as an alternative treatment for atherosclerosis, owing to its ability to inhibit phospholipase A2 by binding its substrate PE. Structural and biochemical studies have shown that PTMs on duramycin are essential for this PE-binding activity. Duramycin exhibits a highly constrained cyclic structure bridged by two thioethers and one unusual lysinoalanine linkage, thus providing a well-formed pocket for PE binding[4]. Additionally, a key 2-hydroxyaspartate (Hya) residue is directed toward the interior of this pocket, where it coordinates the head group of PE. Losses of any of these bridges or of the Hya functionality result in substantial decreases in PE affinity. Although the biosynthesis of thioether formation has been well studied, the enzyme responsible for making the essential lysinoalanine linkage has been less clear.

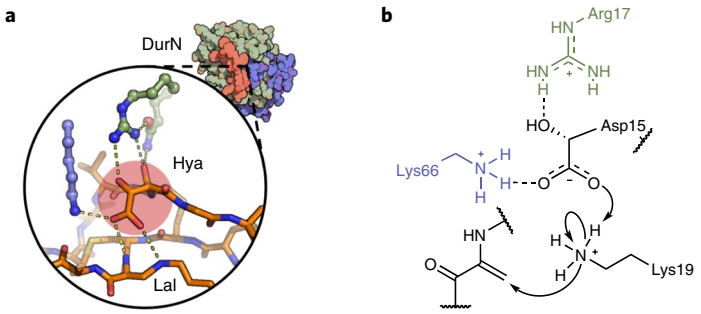

Fig. 1 | Structure and mechanism of DurN-catalyzed lysinoalanine formation. a, DurN binds duramycin at the dimer interface, positioning Hya to make key hydrogen-bonding interactions with the lysinoalanine (Lal) linkage. **b**, Proposed mechanism of Hya-catalyzed Lal formation.

Genetic work previously implicated DurN in lysinoalanine biosynthesis, but DurN shows little or no homology to any known enzyme class[5]. A structure from An et al.[2] now shows that DurN is an interlaced homodimer comprising six α-helices that tightly bind two molecules of duramycin, one at each dimer interface (Fig. 1a). This interaction sets up DurN for substrate-assisted catalysis (SAC) of the lysinoalanine linkage. Specifically, DurN helps to position the Hya residue (formed through hydroxylation of aspartate) in the preduramycin intermediate itself, thus allowing the Hya residue to act as its own catalytic base for conjugate addition of the lysine side chain amino group (Fig. 1b). Mutagenesis and molecular modeling provide further evidence of the essential roles of conformational torsion and Hya positioning in the final maturation step of this peptide natural product. Importantly, this mechanistic strategy allows for the stereochemical outcome of the reaction to be controlled; the researchers show that, although lysinoalanine formation can proceed without DurN under basic conditions, this reaction has no stereoselectivity, and the activity of the product molecule is compromised.

This work suggests a potentially profound role of SAC in RiPP evolution. DurN is not the first example of SAC in an enzyme active site; other key examples include the type II restriction endonucleases and some classes of GTPases, involving pathways in which nucleotide triphosphates are thought to contribute to their own activation[6]. However, DurN is particularly reminiscent of the artificial examples of SAC developed at Genentech some three decades ago, when researchers famously removed the active site histidine from the protease subtilisin and reintroduced it at the P2 position of the peptide substrates[7]. As the researchers wrote in that original paper, "substrates could be distinguished primarily by their ability to actively participate in the catalytic mechanism, permitting the design of extremely specific enzymes." Similarly, the catalytic effect of the Hya in preduramycin ensures selection for products that have this key bioactive feature as well as the optimal tricyclic framework. Clearly, the SAC strategy used in DurN is yet another example of how RiPPs take full advantage of evolution to

home in on exquisitely potent and selective scaffolds. It is likely that more examples of this same powerful logic are waiting to be found in the millions of new biosynthetic pathways pouring out of genome sequencers. ❒

Albert A. Bowers
Division of Chemical Biology and Medicinal Chemistry, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. e-mail: abower2@email.unc.edu

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Competing interests

The author declares no competing interests.