Morphing peptide backbones into heterocycles

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Microbes employ several catalytic strategies to transform conformationally flexible peptide chains into rigidified scaffolds that possess antibiotic or toxin activity. Prominent examples include the biosynthesis of the β-lactam antibiotics of the penicillin and cephalosporin families (1) and the maturation of vancomycin (2) where distinct structural modifications to the nascent peptide chains confer physiological function. In this issue of PNAS, Lee et al. (3) provide the first insight into the chemical structure of streptolysin S (SLS), a hemolytic toxin produced by the human pathogen Streptococcus pyogenes. Its peptide backbone undergoes remarkable posttranslational tailoring, resulting in heterocycle formation and cytolytic activity. Lee et al. further show that a variety of prokaryotes harbor analogous maturation machinery, which suggests widespread use of heterocyclization for altering peptide shape/flexibility and creating functional toxins. This work builds on previous examples where enzymes morph peptide frameworks of both ribosomal and nonribosomal origin.

One famous strategy for constraining peptide flexibility and supplying antibiotic function is the bis-cyclization of the 1,7-diaminomethyl-1-cycloheptane trimeric thiazole moiety (4). Nonribosomal and ribosomal heterocyclic peptides. (A) Isopenicillin N, yersiniabactin, and vancomycin are products of NRPS machinery. Patellamides A and C are ribosomally derived peptides. (B) Conversion of a Gly-Ser-Cys tripeptide into a oxazole-thiazole pair via an oxazole-thiazole intermediate. This two-step process of cyclodehydration and aromatization occurs in microcin, patellamide, and now SLS posttranslational modification.

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flavoprotein-mediated desaturation/ aromatization (Fig. 1). After heterocyclization, removal of the first 26 residues by a signal peptidase occurs. Of the 43 residues present in the mature toxin, 14 of them are used to generate the eight heterocycles, which include a Gly-Ser-Cys moiety that yields an oxazole–thiazole pair. Mature MccB17 targets DNA gyrase and inhibits DNA azole–thiazole pair. Mature MccB17.

The mcbABCD genes, encoding the substrate protein and the three post-translational tailoring enzymes, are clustered. This gene organization is preserved in other systems that morph ribosomal peptides into heterocycles (3, 13). The microcin B17 operon therefore serves as paradigm for several recently discovered examples of ribosomal protein tailoring that occur during maturation.

Of special note is the work of Schmidt et al. (13) to trace the origins of the patel lamides (Fig. 1), heterocycle-containing cyclic peptides, isolated from didemnid extracts. Patellamides A and C are octapeptides that each contain two thiazole and two oxazole rings. They arise from a ribosomally synthesized 71-residue precursor protein, which undergoes prototactropic cleavage, macrocyclization, epimerization, heterocyclization, and dehydrogenation to yield the active cytotoxins. The pat gene cluster, identified during the sequencing of the Prochloron didemni genome, contains seven genes pata–G. Several of these genes encode for the precursor protein (patE), a pro tease (patA) for cleavage of PatE, and tailoring enzymes (patDG) responsible for formation of the thiazole and oxazoline rings from X-Cys and X-Ser dipeptides. The proposed maturation steps, which involve cyclodehydration and aromatization, are reminiscent of those enacted by the mcbBCD gene products in MccB17 maturation. The Schmidt group suggests that the patellamide morphing strategy may be general for the conversion of other cyanobacterial pro-proteins into peptide heterocycles. These analyses augur that other cyclic thiazole-containing peptide antibiot ics, including thiostrepton (14) and GE2270 (15), may arise from similar post-translational modifications.

The contribution of Lee et al. (3) reported in this issue of PNAS can be placed in the context delineated above.

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Their study addresses streptolysin S (SLS), a hemolytic toxin and virulence factor from the human pathogenic bacterium Streptococcus pyogenes, which is responsible for human infections that range from pharyngitis to life-threatening necrotizing fasciitis (16). Despite a long-standing interest in its mechanism of action, the structure of SLS has remained obscure for decades. The current work describes a significant advance, the cloning of a SLS-associated gene locus (17) with the organization sagICBD. Three of the protein products, SagBCD, have homology with McbBCD from the MccB17 gene cluster. SagB shares homology with McbC (flavin-dependent dehydrogenase), SagC with McbB (zinc-dependent cyclodehydratase), and SagD with McbD (ATPase). The sagA gene encodes the 53-residue pro-toxin.

As it happens, the structure of mature SLS remains unknown. Because Lee et al. (3) were unable to detect SagA by mass spectrometry, they used McbA, the 69-residue MccB17 precursor protein, to assay SagBCD activity. The SagBCD complex processes McbA and installs up to four heterocycles into its framework. SagBCD could also convert a maltose binding protein fusion of SagA into a cytolytic product. These studies indicate that SagBCD will convert SagA into a thiazole- and/or oxazole-containing membrane-disrupting toxin. This work sets the stage for the in vitro scale-up and isolation of unfused SLS to determine the number and placement of heterocycles in its peptide backbone.

Bioinformatic analysis of other prokaryotic genomes conducted by Lee et al. (3) indicates the presence of homologous operons in Clostridium botulinum, Listeria monocytogenes, and Staphylococcus aureus RF122, among others. Yet to be elucidated are the circumstances in which these pathways express and morph the pro-proteins into heterocyclic peptides and whether the mature toxins have physiological targets beyond cell membrane disruption (e.g., SLS) and DNA gyrase (e.g., MccB17).

Given the cyanobacterial studies of Schmidt et al. (13) and the current work from Jack Dixon’s group on SLS (3), the posttranslational resculpting of peptide backbones into planar heterocyclic frameworks appears to be much more widespread than initially appreciated. Because some of the predicted gene clusters for heterocyclic metabolites contain additional kinds of tailoring enzymes (e.g., acetyltransferases and methyltransferases) (3), we anticipate that Nature will further morph the peptide frameworks and peripheries to achieve potent and target-specific toxins.

Heterocycles are a recurring motif in Nature’s medicinal chemistry toolbox of bioactive secondary metabolites. Further investigations of the recently uncovered gene clusters for heterocyclic peptide biosynthesis, in addition to the discovery of new tailoring systems, will help elucidate the mechanisms of action of these toxins/antibiotics. The lessons gained from such endeavors will also provide a guide for the combinatorial biosynthesis of novel variants to optimize the future generations of antibiotics.