

Morphing peptide backbones into heterocycles

Christopher T. Walsh* and Elizabeth M. Nolan

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115

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 L- δ -(α -amino adipoyl)-L-cysteinyl-D-valine (ACV) tripeptide to isopenicillin N by isopenicillin N synthetase (IPNS) in penicillin/cephalosporin biosynthesis (1). IPNS, a mononuclear nonheme Fe(II) oxygenase, creates the four-five fused ring system of isopenicillin N (Fig. 1) in one catalytic cycle. Formation of the five-membered thiolane and four-membered β -lactam rings rigidifies the ACV tripeptide scaffold and affords a suicide substrate for peptidoglycan cross-linking transpeptidases that inhibits bacterial cell wall biosynthesis. Further tailoring of the isopenicillin N core provides the various penicillin and cephalosporin family members.

An equally remarkable oxygen-based rigidification strategy occurs in the maturation of vancomycin, which contains a heptapeptide scaffold (Fig. 1) (2). The sequential action of three cytochrome P450-type oxygenases produces the dome-shaped architecture of the cross-linked vancomycin aglycone. The linkages formed include two aryl-ether connections between the side chains of residues 2–4 and 4–6, and a carbon-carbon bond between the aromatic side chains of residues 5–7. Cross-linking of

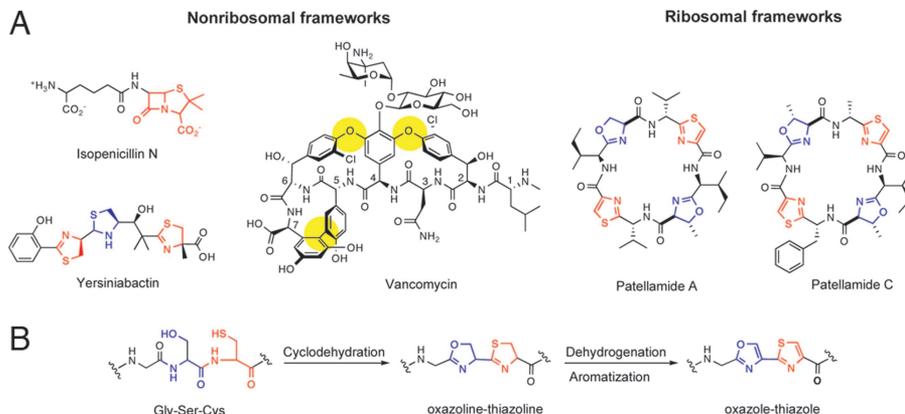


Fig. 1. Nonribosomal and ribosomal heterocyclic peptides. (A) Isopenicillin N, yersiniabactin, and vancomycin are products of NRPS machinery. Patellamides A and C are ribosomally derived peptides. (B) Conversion of a Gly-Ser-Cys tripeptide into an oxazole-thiazole pair via an oxazoline-thiazoline intermediate. This two-step process of cyclodehydration and aromatization occurs in microcin, patellamide, and now SLS posttranslational modification.

the vancomycin heptapeptide is essential for its antibiotic function. The rigidified framework recognizes, binds to, and sequesters the *N*-acyl-D-Ala-D-Ala termini of immature peptidoglycan strands, and blockade of bacterial cell wall biosynthesis results.

The penicillins, cephalosporins, and vancomycins are prominent members of the nonribosomal family of antibiotics. They are synthesized in the cytoplasm on nonribosomal peptide synthetase (NRPS) assembly lines. Their amino acid sequences are determined by multi-modular protein thiotemplating rather than by mRNA (4). Another hallmark of nonribosomal peptide synthesis logic is the heterocyclization of X-Cys and X-Ser dipeptide moieties, which occurs during thiotemplated peptide chain elongation. Cyclodehydration of X-Cys and X-Ser dipeptides forms thiazolines and oxazolines, respectively, and also rigidifies the peptide backbone. The five-membered thiazoline and oxazoline rings are commonly found in siderophores, high-affinity Fe(III) chelators produced by some periods during periods of nutrient deprivation, where they provide (now) basic nitrogen donor atoms for Fe(III) coordination (5, 6). Further enzymatic tailoring of such cyclized peptide backbones alters their redox state and function. For instance, reduction of thiazoline to thiazolidine occurs in the maturation of the siderophore yersiniabactin (Fig. 1) (7), and oxidation of thiazoline rings to planar aromatic thiazoles occurs in the tailoring of the

antitumor antibiotic bleomycin (8). During its maturation, a Cys-Cys residue pair is cyclodehydrated and oxidized to a bithiazole moiety, which is a DNA intercalator. Resculpting of the Cys-Cys dipeptide into the planar, intercalating bithiazole constitutes remarkable re-engineering of the bleomycin peptide backbone.

Cyclodehydration of X-Cys and X-Ser peptide linkages is not restricted to peptides synthesized on NRPS assembly lines. Prokaryotic ribosomal protein products can undergo the same types of modification. The best-studied example of a ribosomal protein-to-heterocycle morphing system is the posttranslational modification of McbA, a 69-residue pro-toxin produced by some types of enterobacteria, which affords the peptide toxin microcin B17 (MccB17) (9–12). Three tailoring enzymes, McbBCD, modify the nascent peptide and create four thiazole and four oxazole moieties from six glycines, four serines, and four cysteines. *In vitro* characterization of the McbBCD proteins verified cyclodehydration and desaturation activity, and validated the two-step process of Gly-Cys/Gly-Ser dipeptide cyclodehydration followed by

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*To whom correspondence should be addressed. E-mail: christopher.walsh@hms.harvard.edu.

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