PEPTIDE MACROCYCLIZATION

Cycling back to biocatalysis

Macrocyclic peptide natural products are important medicinal compounds. The catalytic properties of an unusual peptide cyclase enzyme have recently been described — providing opportunities for the engineering and synthesis of structurally complex peptides with novel biological activities.

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Macrocyclic peptide natural products such as vancomycin and cyclosporin have enjoyed widespread medicinal use because of their potent biological activities. The reduced conformational flexibility afforded by the peptide macrocycle typically provides high-affinity target binding and enhanced proteolytic stability. These features have made macrocyclic peptides intriguing targets for the development of novel pharmaceuticals[1–3].

Despite this promise, controlling the efficiency and regiospecificity of peptide macrocyclization remains challenging for synthetic chemistry[4]. Thus, researchers are increasingly turning to macrocyclic peptide biosynthetic enzymes, which often provide exquisite stereo- and regiochemical control over the macrocyclization and are amenable to engineering and synthetic scale-up using fermentation processes. Now, writing in *Nature Catalysis*, Ikuro Abe, Toshiyuki Wakimoto and co-workers report mechanistic and structural studies of a recently identified[5] novel cyclase enzyme involved in the biosynthesis of surugamides[6] (Fig. 1) — non-ribosomal peptide (NRP) natural products produced by *Streptomyces* sp. JAMM992.

NRPs are natural products produced by modular enzymatic assembly lines called NRP synthases (NRPS)[7,8], multifunctional enzymes composed of modules that are distributed over several polypeptides. For example, the surugamide A assembly line

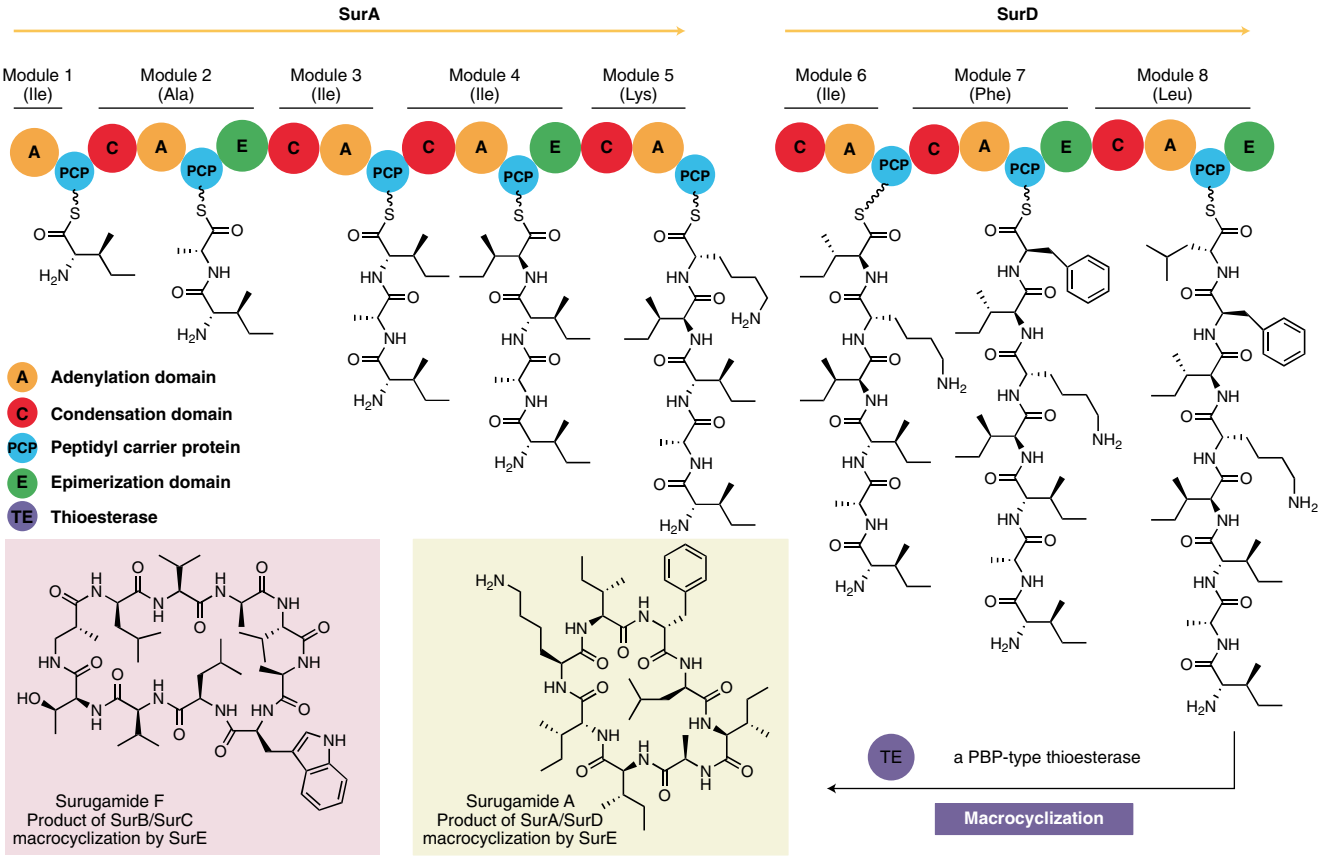

Fig. 1 | Overview of surugamide A biosynthetic pathway. The surugamide A non-ribosomal peptide synthtase (NRPS) assembly line encompasses eight modules distributed over two polypeptide chains (SurA and SurD). Each module is responsible for incorporating a specific amino acid into the growing peptide chain, which is covalently tethered to the assembly line. In the final step, the nascent peptide is offloaded from the assembly line by an unusual PBP-type thioesterase enzyme (TE, SurE) which concomitantly macrocyclizes the peptide chain into surugamide A. An analogous NRPS assembly line consisting of SurB/SurC functions in concert with SurE to produce the cyclic decapeptide, surugamide F.

shown in Fig. 1 contains eight modules distributed over two polypeptides (SurA and SurD). Each module contains an adenylation (A) domain that catalyses the aminoacylation of the peptidyl carrier protein (PCP) domain of that module, covalently tethering the peptide to the enzymatic assembly line. A condensation (C) domain then catalyses peptide bond formation between the activated PCP-aminoacyl thioester and the PCP-peptide thioester on the upstream NRPS module to extend the C terminus of the peptide chain by one amino acid. Additional tailoring enzymes (such as the epimerization (E) domains in modules 2, 4, 7 and 8 of the surugamide NRPS) expand the structural diversity of the NRP. Ultimately, the nascent peptide is released from the terminal module of the assembly line by a thioesterase (TE) enzyme, many of which catalyse the concomitant macrocyclization of the peptide. TE enzymes can either be incorporated into the terminal NRPS module (*cis*-acting TEs) or they can be stand-alone enzymes (*trans*-TEs) that interact non-covalently with the assembly line.

Previous work established that the surugamide biosynthetic gene cluster was devoid of canonical *cis*- or *trans*-acting TE domains, but instead encoded for an enzyme that shares homology with penicillin binding proteins (PBPs). Biochemical work confirmed that this enzyme (SurE) was a novel *trans*-acting TE that catalysed the cyclization of the surugamides, establishing a new class of peptide cyclase enzymes (subsequently dubbed the PBP-type TEs)[5].

The in vitro kinetic analysis reported by Ikuro Abe co-workers revealed the strict stereoselectivity of SurE towards peptidyl substrates with an L-amino acid at the N terminus and a D-amino acid at the C terminus[5]. Strikingly, the canonical *cis*-acting TE domains were found to have opposite stereochemical requirements (a D-amino acid at the N terminus and an L-amino acid at the C terminus). SurE also exhibited flexibility towards the amino acid side chains at the N- and C-terminal positions, and was found to catalyse cyclization of derivatives containing alanine substitutions at interior positions of the peptide in quantitative yields with no detected formation of linear side products. This impressive cyclization fidelity with non-natural analogues should prove to be a useful property for biocatalytic applications.

To understand the structural basis for catalysis in more detail, the researchers solved the X-ray crystal structure of SurE to 2.2 Å resolution, revealing a bi-domain structure consisting of an N-terminal α/β-hydrolase fold similar to other β-lactamase PBPs[9], and a C-terminal lipocalin-like domain containing eight antiparallel β-strands. The active site of the PBP domain contained the catalytic nucleophile (Ser63) and other conserved residues needed for transfer of the peptide chain from the NRPS PCP domain to the SurE-Ser63 nucleophile. The C-terminal lipocalin domain stacks on the PBP domain to form a cleft that is likely to facilitate peptide binding and guides intramolecular macrocyclization to release the cyclic peptide from the SurE-Ser63 nucleophile. Consistent with this chemical mechanism, soaking of the *apo*-SurE crystals with a cyclization-deficient peptide substrate resulted in covalent tethering of the peptide to Ser63 and concomitant disordering of a nearby flexible loop in SurE (residues 211–224). Overall, the structural data suggest that SurE is likely to require significant conformational changes to accommodate recognition of the NRPS assembly line, to catalyse peptide transfer from the assembly line to the Ser63 nucleophile, and to catalyse peptide macrocyclization.

One unusual feature of the surugamide biosynthetic gene cluster is that it encodes two separate NRPS assembly lines (SurA/SurD and SurB/SurC), which produce two separate NRPs (surugamides A and F, respectively, Fig. 1), both of which are cyclized by SurE. This suggests that SurE possesses an innate ability to interact with the PCP domains of multiple assembly lines. To explore the in vivo engineering potential of SurE in more detail, the authors genetically disrupted modules 5 and 6 in the *surC* gene in the *Streptomyces* native producer. Astonishingly, this engineered strain produced a new cyclooctapeptide (rather than the typical cyclodecapeptide, surugamide F), suggesting that SurE successfully intercepted and cyclized the NRP from the non-cognate PCP domain in module 4 of SurC. Finally, bioinformatic analysis revealed more than 200 SurE homologues clustered with NRPS genes in bacterial genomes. These PBP-type TEs fall into two classes according to the presence (class I) or absence (class II) of the C-terminal lipocalin domain. Remarkably, two recombinant class I SurE homologs of unknown function discovered by this study were expressed, purified, and shown to catalyse cyclization of surugamide-like peptide thioesters in vitro, suggesting that substrate promiscuity might be a general feature of PBP-type TE cyclases.

Overall, the report by Ikuro Abe and co-workers provides detailed mechanistic and structural insights into the recently discovered PBP-type TE enzyme family and highlights the potential of these enzymes for the engineering of macrocyclic NRPs. Namely, the study reveals not only the strict stereoselectivity of the terminal amino acids, but also the broad substrate specificity of several PBP-type TEs. More encouragingly, the research illuminates the ability of PBP-type TEs to offload and cyclize non-native PCP-peptidyl substrates in vivo, opening the door to future metabolic pathway engineering and synthetic biology applications. Moving forward, while the modular NRPS assembly lines have intrigued bioengineers for quite some time, NRPS engineering remains in its infancy, and extensive effort will be required to increase the catalytic efficiency and biosynthetic fidelity of engineered NRPS systems. Along these lines, more detailed investigation of the interesting structural dynamics of SurE and other peptide cyclases — and of how the conformational properties of these enzymes relate to their biochemical functions — should help to inform future engineering efforts. Nevertheless, SurE promises to be a useful catalyst in the toolbox for macrocyclic peptide synthesis and engineering. ❐

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Competing interests

The authors declare no competing interests.