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Daptomycin Structure and Mechanism of Action Revealed

Daptomycin kills otherwise antibiotic-resistant gram-positive pathogens and is the first lipopeptide antibiotic to reach the clinic. Elucidation of its 3D structure and mechanism of action, reported in this issue of *Chemistry & Biology* [1], will facilitate the design and engineering of new, potentially life-saving antibiotics.

The calcium-dependent antibiotic daptomycin recently received approval for clinical use. Daptomycin is a tridecapeptide comprising several nonproteinogenic amino acids with an N-terminal decanoyl fatty acid side chain and a decapeptide lactone core resulting from the cyclization of the Thr-4 hydroxyl group onto the C-terminal carboxylate (Figure 1). Daptomycin is produced as a complex mixture of lipopeptides by *Streptomyces roseosporus* [2] and shares a similar structure, and possibly a related mode of action, to other acidic lipopeptide antibiotics. These include the calcium-dependent antibiotics (CDA) from *Streptomyces coelicolor* [3], A54145 from *Streptomyces fradiae* [4], and the friulimicins along with amphomycins from *Actinoplanes friuliensis* [5]. Notably, all of these antibiotics possess N-terminal fatty acid moieties and decapeptide lactone or lactam rings containing several acidic residues, which are important for calcium binding and antibacterial activity. Recently, scientists at Ecopia Biosciences Inc. have used a genomics-based approach [6, 7] to identify numerous previously uncharacterized lipopeptide biosynthetic gene clusters, suggesting that many more natural lipopeptide antibiotics have yet to be isolated.

The acidic lipopeptides belong to the nonribosomal peptide family of natural products. Nonribosomal peptides are among the most structurally diverse and widespread secondary metabolites in nature and include important therapeutic agents, such as vancomycin, cyclosporin, and bleomycin, as well as daptomycin. Given their structural complexity, total synthesis is usually unable to provide the quantity or diversity of products required for drug development. As a result of this, there has been considerable effort aimed at understanding how these molecules are biosynthesized, with a view to developing methods that will allow for the engineered (or combinatorial) biosynthesis of novel variants [8, 9]. Central to the biosynthesis of these peptides are the nonribosomal peptide synthetases (NRPS). These large assembly-line enzymes contain multiple modules, each of which is responsible for the activation and incorporation of a specific amino acid into the growing peptide chain. Using a combination of exquisite structural biology and enzymology, the function and substrate specificities of the individual catalytic domains within NRPS modules have been elucidated [9]. Consequently, methods have been developed which have enabled the engineered biosynthesis of new products. For example, Marahiel and coworkers have surgically replaced domains within NRPS modules or deleted whole modules by manipulation of the NRPS encoding genes responsible for the biosynthesis of the cyclic lipopeptide surfactin in *Bacillus subtilis*. The resulting mutant strains produce new surfactin derivatives of different amino acid sequence and ring size [10, 11]. The key specificity-conferring domain within an NRPS module is the adenylation (A) domain, which activates the substrate amino acid, forming an amino acyl-AMP intermediate, prior to covalent attachment to the NRPS and peptide bond formation. The same group has also shown that, by changing as few as one amino acid residue at the active site of an A domain, it is possible to change the specificity of the A domain and generate a surfactin derivative with altered sequence [12].

Biosynthetic engineering of nonribosomal peptides is achieved by reprogramming the genes that encode the key biosynthetic enzymes. It is therefore essential that the biosynthetic gene cluster is first isolated, cloned, and then sequenced. The recently completed *S. coelicolor* genome-sequencing project revealed the first entire biosynthetic gene cluster of an acidic lipopeptide antibiotic, CDA. Using this genetic template, it was possible to engineer the biosynthesis of new CDAs using a mutasynthesis approach [3]. To achieve this, a gene required for the biosynthesis of one of the unusual amino acids in CDA, hydroxyphenylglycine (HPG), was deleted from the gene cluster. Feeding synthetic analogs of HPG and its precursors resulted in CDAs with phenylglycine or 4-fluorophenylglycine residues in place of HPG. Further attempts to engineer CDA have focused on active site modification of A domains [13]. Changing two residues within one of the key Asp-activating A domains resulted in CDA containing Asn instead of Asp at position 7. Significantly, the new peptide is no longer bioactive, suggesting that Asp-7 is essential for calcium binding and activity. Noticeably, yields of the new Asn-containing CDA were reduced, and significant quantities of the CDA-hexapeptide intermediate were isolated, presumably due to hydrolysis by an unidentified NRPS proofreading activity. An elegant complementary chemoenzymatic approach has also been used to generate CDA-like peptides. Here, the CDA NRPS thioesterase domain (or peptide cyclase) was used in vitro to cyclize a number of synthetic CDA-like linear peptides with C-terminal thioesters [14]. Cubist Pharmaceuticals, who produce daptomycin under the trade name Cubicin, have also completed sequencing the daptomycin biosynthetic gene cluster [15]. This will greatly facilitate their biosynthetic engineering efforts toward second-generation lipopeptide antibiotics (dapt-II).

Daptomycin exhibits bactericidal activity against life-threatening pathogens that are resistant to all current treatments, including vancomycin, and represents the first new class of natural antibiotic to reach the clinic in many years. Not surprisingly, its arrival was met with considerable interest and optimism [16]. Currently, dap-

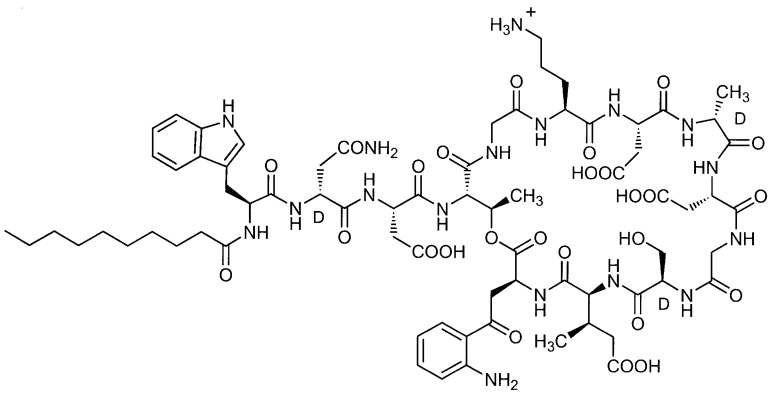

Figure 1. The Structure of Daptomycin

tomycin is being used to treat skin infections, but trials are underway for its use to combat more dangerous life-threatening infections including endocarditis. This is despite the fact that until now daptomycin's 3D stucture remained unknown, and its distinct mechanism of action was the subject of considerable debate. However, in this issue of *Chemistry & Biology*, Hancock and coworkers [1] describe the significant advances that they have made toward answering these key questions. Using a combination of CD and fluorescence spectroscopy, they show that daptomycin undergoes significant calcium-dependent conformational changes upon association with model lipid membranes. Once inserted into the membrane, significant perturbations, including lipid flip-flop and membrane leakage, ensue. Detailed NMR studies revealed for the first time the 3D structure of both free and calcium-bound daptomycin. This showed that the binding of calcium ions causes the core decapeptide lactone to draw inwards, with Gly-5 functioning as a flexible hinge, probably allowing Asp-3 and 7 to coordinate calcium. In addition to reducing the charge and constraining the conformational freedom of the peptide core, calcium binding increases the amphipathicity and solvent-exposed hydrophobic surface. This not only facilitates penetration into the membrane but also results in oligomerization of the peptide. Finally, Jung et al. [1] provide evidence that membrane depolarization occurs after cell death and is a consequence of, rather than a cause of, the bactericidal activity of daptomycin. As a result of this work, the earlier model describing the mechanism of action of daptomycin, proposed by Silverman et al. [17], has been refined.

While the exact nature of the molecular targets of daptomycin within the cytoplasmic membrane have yet to be established, some of the key structural requirements for activity have been revealed. It is essential that we continue to probe the relationship between structure and mechanism of action, not only for daptomycin but also for the other acidic lipopeptide antibiotics. Only when armed with this vital knowledge will it be possible to design new and improved lipopeptide antibiotics that can be produced using the innovative methods of biosynthetic engineering described here. Clearly, we are in a race against time with life-threatening pathogens that will eventually evolve resistance to even daptomycin. Fortunately, our ability to decipher how these complex antibiotics work [1, 17] and our capability to engineer more potent variants [3, 8–14] should ensure that we will win.

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