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Enzyme-Assisted Antibiotic Engineering—the Wright Way

Wright and coworkers describe the design, synthesis, and screening of cyclic peptides with chimeric structures that draw from two disparate antibiotics, tyrocidine and a type B streptogramin [1]. An active compound identified appears to function by a different mechanism of action than the parent antibiotics and also overcomes resistance to type B streptogramins.

Metabolic engineering is frequently provided as an ultimate goal behind natural product biosynthetic pathway research. While considerable development has been made both in academia and industry, it is clear that polyketide and nonribosomal peptide synthase engineering will require significant development before it becomes widely used as a synthetic technique. Luckily, natural product pathway elucidation also provides the chemist with new tools for chemoenzymatic processes [2]. Using enzymes with broad substrate specificity, chemists may capitalize upon the chemo-, regio-, and stereospecificity of enzymatic catalysis. The selective choice of specificity provides further versatility to synthetic chemistry. Wright and coworkers demonstrate that enzymes may be used not merely as tools for process chemistry, but they may also serve as robust catalysts for drug discovery.

Nonribosomal peptide synthases (NRPSs) produce a diverse array of therapeutic molecules, including the clinically important antibiotics penicillin, vancomycin, bacitracin, gramicidin S, and streptogramin. Due to the emergence of antibiotic-resistant bacterial strains and the fact that natural product screens now return over 99% known compounds [3], a fusion of organic synthesis, molecular engineering, and natural product chemistry is currently being called upon to fill a growing need for new antibacterials. Mukhtar, Koteva, and Wright [1] report a synthesis relying on all three disciplines. They start with two antibiotics, one of which is semisynthetic, and merge them using solid-phase peptide chemistry and catalytic cyclization with the macrocycle-forming thioesterase (TE) domain from Tyrocidine C (TycC) biosynthesis. The use of TycC TE for chemoenzymatic synthesis on solid phase is by now well established [4]. This enzyme has been used in the recent past for the creation of small cyclic peptides (6–14 amino acids) and for the synthesis of peptide-polyketide hybrids and glycopeptides [5–7].

Streptogramin antibiotics are classified either as type A, cyclic peptide-polyketide macrolactones, or type B, cyclic depsipeptides. Both classes act on the bacterial ribosome, with type A binding the peptidyl transfer center and type B blocking the peptide exit tunnel [1]. Type A and B streptogramins also act synergistically, where binding of a type A compound facilitates the binding of a type B compound. Although known for over 50 years, streptogramin antibiotics were only recently approved for human use [8]. Synercid, introduced by Rhone-Poulenc in 1999, is a mix of dalfopristin (type A) and quinupristin (type B) streptogramins. Perhaps because of their long use in agriculture, streptogramin B resistance is already widely reported and occurs through one of three mechanisms: efflux by Msr pumps, methylation of 23S rRNA by Erm, or cleavage of the cyclic ester by Vgb. The authors reason that, if the vulnerable ester in type B streptogramins were replaced with a more stable amide linkage, the new compound would be impervious to the lyase action of Vgb. They develop a strategy using chemoenzymatic synthesis to create a chimeric antibiotic. In so doing, the depsipeptide ester is replaced by an amide using solid phase peptide synthesis and the TycC TE enzyme as a catalyst. This enzyme has been shown to be promiscuous in cyclizing a number of small peptides when the first amino acid contains an aromatic ring and the penultimate residue contains a cationic amino functionality [4]. Using this approach, the authors create a collection of compounds containing both the active portions of quinpristin (a type B streptogramin) and the moieties of tyrocidine required for cyclization. By preserving biologically active portions of both starting compounds, the authors propose a rational approach to design chimeric antibiotics.

These studies identified several compounds demonstrating antimicrobial activity without susceptibility to Vgb hydrolysis. The authors chose one top candidate for further characterization (compound **5**) and report that it demonstrates an MIC range from 32 to 256 μg/ml against gram-positive bacteria. Most surprisingly, the compound seems to act through an unusual mechanism of action. Compound **5** is neither a substrate for Vgb nor an inhibitor of it—evidence that the conserved ester in type B streptogramins is required for Vgb binding. This information alone may prove useful for future attempts to design streptogramin analogs. The molecule was not synergistic with dalfopristin (a type A streptogramin), nor did it interact with the ribosome. In addition, the compound was not active in disrupting membranes, which is the mode of action for tyrocidine. Taken together, these results are quite remarkable: the authors have created a chimeric antibiotic that overcomes known resistance mechanisms and appears to have a mode of action separate from its parent compounds.

This report illustrates the advantages in combining organic synthesis with natural product biosynthetic enzymes to further drug discovery. While the authors call this serendipity, it is more likely a case of chance favoring the prepared mind. Nature has spent millions of years evolving active motifs into natural products, so it is perhaps not surprising that, by retaining the active portions of the two natural products, a new—or previously undiscovered—biological interaction was uncovered. This idea would follow the discovery by Kahne

and coworkers in which vancomycin was shown to demonstrate dual activities inhibiting separate peptidoglycan biosynthetic enzymes at opposite sites of the molecule [9].

This work also raises the prospect of creating a library of TE domains as a toolkit for molecular discovery. What sort of previously undiscovered reactions and molecules would then be available? And if a molecule is discovered in this way and proves to be a useful clinical agent, could large-scale production of these peptides be achieved? We turn again to the prospect of metabolic engineering, which may be the method by which to produce compound **5** on a process scale.

Andrew C. Mercer and Michael D. Burkart
Department of Chemistry and Biochemistry
University of California, San Diego
9500 Gilman Drive
La Jolla, California 92093

Selected Reading

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