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Current Opinion in Chemical Biology

Structure guided approaches toward exploiting and manipulating nonribosomal peptide and polyketide biosynthetic pathways

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Nonribosomal peptide and polyketide natural products are structurally diverse small molecules synthesized on complex enzyme assemblies. The ability to rationally engineer secondary metabolic pathways is a promising approach to novel therapeutics. Atomic resolution structures of biosynthetic enzymes provide information on active site architecture and macromolecular assembly that can aid in the engineering of new compounds. This review surveys recent applications toward biosynthetic engineering of natural products guided by structural biology.

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Current Opinion in Chemical Biology 2012, **16**:162–169

This review comes from a themed issue on
Biocatalysis and Biotransformation
Edited by Jon S Thorson and Ben Shen

Available online 25th February 2012

1367-5931/$ – see front matter

DOI 10.1016/j.cbpa.2012.02.002

Introduction

The rational manipulation of secondary metabolite pathways is a promising route to novel therapeutics and biological probes [1]. Polyketide (PK) and nonribosomal peptide (NRP) natural products (Figure 1) are biosynthesized on large, multimodular protein assemblies responsible for the oligomerization of precursors [2]. Several characteristics of PK synthases and NRP synthetases have made these systems attractive targets for rational manipulation [3]. These include the frequent co-linear relationship between the primary genetic sequence and the produced natural product and the ability to predict the composition and structure of the product based on analysis of the gene cluster. Detailed structural characterization of key enzyme players would facilitate an engineering approach by identifying the determinants for substrate specificity, providing a basic understanding of the chemistry and describing protein/protein interfaces between domains [4]. The following review examines recent approaches that apply structural biology to aid in the manipulation of biosynthetic pathways.

Nonribosomal peptide biosynthesis

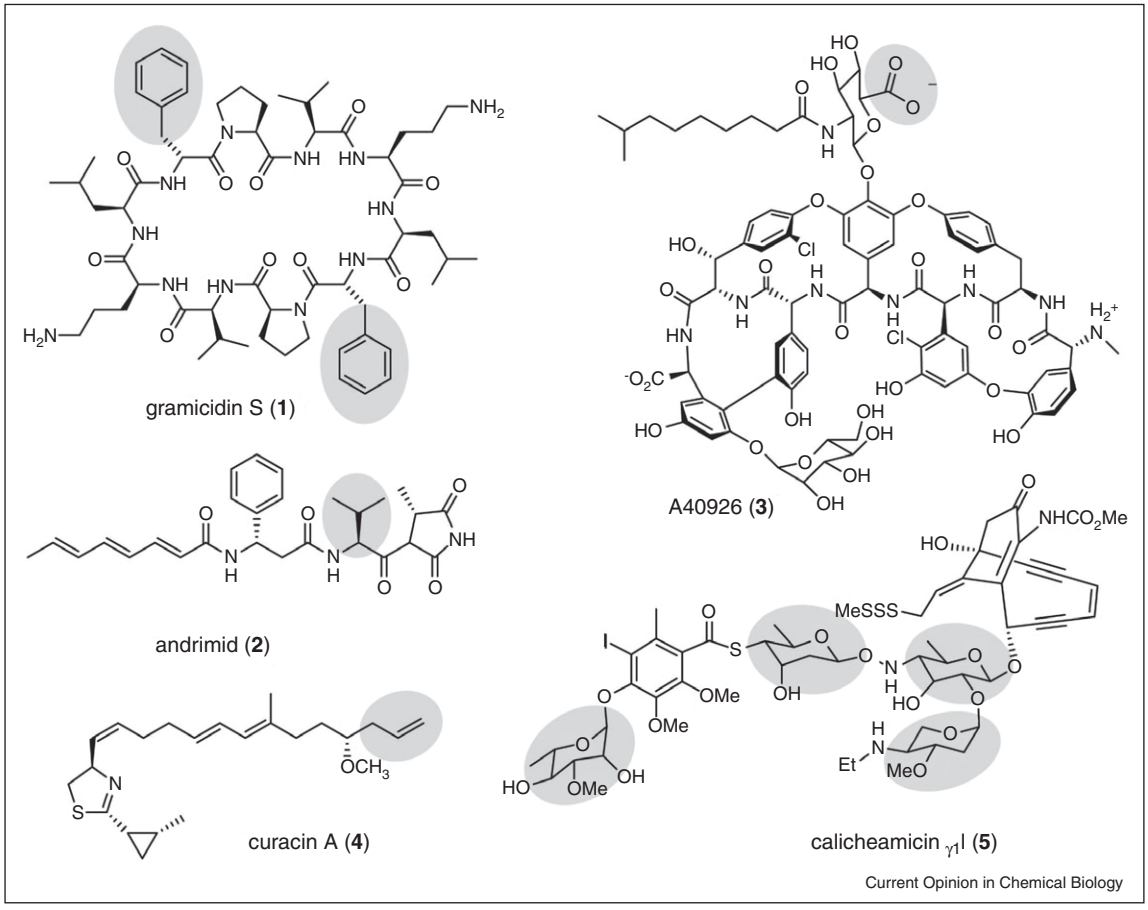
The NRP family of natural products includes a structurally diverse array of important therapeutics and biologically active small molecules [5]. NRPs are distinguished by the wide array of amino acids incorporated into the natural products, often distinct from the proteinogenic amino acids. The precursors are oligomerized, commonly via amide linkages, on modular NRP synthetase assemblies through a thioester-templated mechanism common also to PK and fatty acid (FA) synthases. Additionally, the peptidic product is frequently subject to further enzymatic transformations (tailoring) leading to the ultimate natural product.

Precursor biosynthesis in nonribosomal peptide pathways

Studies into the biosynthesis of amino acid based precursors in NRP pathways facilitates approaches to precursor-directed biosynthesis of natural products and the production of chiral synthons for use in chemical synthesis. β-amino acids are important components of many biologically relevant natural products [6,7]. There are several routes in the primary and secondary metabolism of bacteria to β-amino acids [8–10]. The recently described MIO (4-methylideneimidazole-5-one)-dependant aminomutases interconvert α-aromatic and β-aromatic amino acids through a cinnamate intermediate [11–14]. X-ray structures of L-Tyr aminomutase (TAM) and L-Phe aminomutase (PAM) have recently been solved [15–17]. Aminomutases are characterized by a K_{eq} of ~1 (α-amino acid/β-amino acid) and as the β-amino acid is often the more desirable synthetic product, this is not ideal. To address this, Janssen and co-workers generated a series of mutants in PAM that resulted in increased β-regioselectivity while retaining high activity and enantioselectivity [18••]. A laboratory scale reaction with one of these mutants (Q319M), *trans*-cinnamic acid and ammonia gave a final yield of 50% with a 7:1 ratio of β-amino acid to α-amino acid. This work gives evidence that the regiospecificity of aminomutases can be altered to yield a higher percentage of a desired synthetic product.

Although wt-PAM is highly enantioselective with a wide substrate scope, the same is not true for many characterized TAMs [19–21]. Attempts to increase the selectivity of TAM through a similar mutagenesis strategy as above only produced kinetically sluggish enzymes [22,23]. The same group also set out to engineer a regioselective and enantioselective TAM from *T. chinensis* PAM by altering substrate specificity [24]. Sequence

Figure 1



Selected NRP and PK natural products. Example nonribosomal peptides and polyketides discussed in the text. The area of the natural product that is the focus of structural biology and/or biosynthetic engineering is highlighted.

alignments of aminomutases revealed a conserved His residue in TAM that is frequently a Phe in PAM enzymes. Mutation of this residue in *T. chinensis* PAM did allow conversion of L-tyrosine to β-tyrosine, but with poor enantioselectivity. Further studies revealed a Ser mutant at this position satisfied both criteria for a moderately active enzyme with high regioselectivity and enantioselectivity, overall, providing evidence that inherently promiscuous enzymes are excellent tools for developing altered substrate specificity and reactivity.

Adenylation domain of NRP synthetases

The modular nature of NRP synthetases creates a unique platform for the genetic engineering of novel structures [3]. Adenylation (A) domains are the primary determinant of amino acid specificity. The first structure of an A-domain, the L-Phe specific domain from gramicidin (**1**) synthetase (GrsA), was solved in 1997 [25]. Based on the significant structural homology among A-domains, a specificity conferring 'code' was developed based on primary sequence analysis [26,27]. In general, efforts to alter substrate specificity through site-specific mutations based on the 'code' or swapping intact domains has been met with mixed results [28–31]. Recent advances in computational algorithms have been applied to the redesign of A-domains for alternate substrates. These methods allow for the rapid prediction of specific mutations within the code region and elsewhere in the protein that can lead to a substrate switch. Chen *et al.* used GrsA as a model and found a triple mutant with significantly enhanced affinity for an alternate substrate (L-Leu) versus the natural substrate (L-Phe) [32•]. This work validates computational approaches to A-domain redesign and gives the groundwork for further studies into the effects of mutations outside the A-domain including the PCP-domains and C-domains. An alternative approach involved *in vivo* generation of an A-domain mutant library of the three variant residues in the code region (AdmK, a valine specific A-domain in the andrimid (**2**) pathway) [33•]. Several mutants were found to give analogs of andrimid at the position of L-Val. The most efficient of these was a quadruple-mutant containing a serendipitous