Chemistry & Biology, Vol. 9, 1163–1166, November, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S1074-5521(02)00267-3

Previews

New Compounds By Combining "Modern" Genomics and "Old-Fashioned" Mutasynthesis

Molecular approaches enable the identification of genes that operate in natural product biosynthetic pathways. Microbes containing mutations in those genes can be fed alternative precursors, generating derivatives of complex natural products, which may not be obtainable by chemical methods.

Natural products exhibit an enormous source for drug leads, which is exemplified by the finding that 60% of all new antibacterial and anticancer drugs approved between 1983 and 1994 were derived from natural compounds [1]. However, before these natural compounds are commercially developed, they usually have to be modified to generate a collection of chemically related structures. Optimization of these drug leads can be achieved using modern organic chemistry, but this approach is limited for complex, high molecular weight natural products that contain a great number of reactive groups, some of which require protection during the reaction process. Even though Nicolaou and others have convincingly demonstrated that highly complicated molecules such as vancomycin, everninomycin, or epothilone can be synthesized de novo [2], alternative techniques are required for the production of the larger amounts of compounds that are needed for pharmaceutical purposes.

In 1985, Hopwood and colleagues demonstrated a new approach for lead optimization by using genes from different natural product-producing microbes to create novel hybrid compounds [3]. Madduri and coworkers proceeded to adapt this combinatorial biosynthetic approach for an industrially relevant compound in 1998 [4]. By introducing a desoxysugar biosynthesis gene into the doxorubicin producer *S. peucetius,* the authors generated a strain that directly synthesized one of the most important antitumor drugs, epirubicin (4′-epidoxorubicin). Over last few years, combinatorial biosynthesis has been shown to be a powerful tool for modifying several natural compounds with highly diverse structure [5].

An alternative technique, which has also widely been used, is to feed altered natural product precursors to microorganisms, which may subsequently be incorporated in a product of interest. However, one drawback of this approach is that since, under normal circumstances, the alternative precursors must compete with the natural precursors, the yield of novel derivatives is rather low. This disadvantage can be overcome by blocking the synthesis of the natural precursor, either by mutating key genes [6] in the respective synthetic pathway or by adding specific inhibitors of biosynthetic enzymes.

To date, these manipulations were rather heavy handed, since the inhibitors often influence other parts of the pathway, and the mutations were obtained by chemical or UV mutagenesis, which led to additional unwanted mutations in other genes. Crucially, the tremendous progress that researchers have achieved in elucidating and manipulating the biosynthetic pathways of secondary metabolites can now be used to make "surgical" interventions which affect only one desired pathway, specifically blocking the production of a certain building block or structural feature of a particular corresponding compound. Thus, genetics can be used to generate mutants where the synthesis of a specific precursor is prevented, and these mutant strains can subsequently be used for mutasynthesis, a better term for which may be "precursor-directed biosynthesis."

The use of precursor-directed biosynthesis promises to be particularly useful for modifying the chemical backbones of highly complex molecules like the pharmaceutically important type I polyketides (PKS) (e.g., erythromycin) or nonribosomally synthesized peptides (e.g., vancomycin). The backbones of both of these compounds are synthesized in an assembly-line manner by multimodular enzymes and are subsequently modified by tailoring enzymes [7]. Although the application of precursor-directed biosynthesis has been demonstrated for PKS-type antibiotics [8], such experiments have not been reported for nonribosomally synthesized peptides until recently.

An interesting feature of metabolites that are synthesized by nonribosomal peptide synthetases is that they often contain nonproteinogenic amino acids. Recently, some of the mechanisms for syntheses of amino acids such as 3,5-dihydroxyphenylglycine (DPG) [9], β-hydroxylated amino acids [10, 11], and 4-hydroxyphenylglycine (HPG) [12] have been elucidated, and the genes involved in the synthesis of these and further nonproteinogenic amino acids have been identified and functionally characterized. These genes are part of a particular biosynthetic gene cluster. This information make it easier for researchers to analyze new biosynthetic gene clusters: after a cluster has been identified and the putative function has been assigned in silico by using bioinformatic tools, the respective genes can easily be identified and directly be cloned for manipulation. This knowledge has now been used to engineer nonribosomally synthesized peptide antibiotics by targeted mutation and precursor-directed biosynthesis [13] (Hojati et al., this issue [19]).

Two groups lead by Smith and Micklefield report the generation of novel CDA (calcium-dependent antibiotic) derivatives. It is well established that CDA is produced by the model streptomycete *S. coelicolor* [14]. CDA belongs to the group of acidic lipopeptide antibiotics that consist of 11 amino acids to which a lipid part has been attached [15]. Although the accurate target of CDA is not yet known, antibiotics showing structural similarity, like daptomycin or friulimicin, are of great importance because they potently inhibit bacterial cell wall synthesis. Albeit undefined, this unique mechanism of action is the reason that CDA-related drugs may become so important in treating infections from severe antibiotic resistant pathogens, such as methicillin-resistant *Staphylococcus aureus* strains (MRSA) and vancomycin-resistant enterococci (VRE).

The genes for CDA biosynthesis are localized to an 82 kb region of the *S. coelicolor* genome. The sequence of the CDA cluster [16, 17] was used in silico to determine a putative biosynthetic pathway, which correlates well with the recently suggested functions for these genes [18] (the authors could not assign a function for some genes). Although the authors provide no biological evidence for the complete hypothesized pathway, there is no doubt that the crucial steps were correctly predicted.

CDA contains (beside others) the crucial nonproteinogenic amino acid L-4-hydroxyphenylglycine (HPG). This amino acid is also present in the backbone of peptides (complestatin and nocardicin), glycopeptides, (vancomycin and teicoplanin), further lipopeptides (arylomycin), and the lipoglycodepsipeptide antibiotic ramoplanin. The HPG synthesis requires as the key step the action of a 4-hydroxymandelate synthase (HmaS), catalyzing the decarboxylation and hydroxylation of 4-hydroxyphenylpyruvate leading to 4-hydroxymandelate [12]. Two additional enzymes, the 4-hydroxymandelate oxidase (Hmo) and the 4-hydroxyphenylglycine transaminase (HpgT), complete the HPG biosynthesis.

Hojati et al. inactivated the *hmaS* gene of the CDA gene cluster, generating a mutant that is blocked in HPG biosynthesis and is therefore unable to synthesize CDA. Subsequently, the authors fed a series of synthetic mandelate, arylglyoxylate, and arylglycine analogs to obtain new CDA derivatives by precursor-directed biosynthesis. In conclusion, by feeding the mutant cells 4-fluoro and 4-dehydroxy analogs, novel CDA peptides were synthesized, and the structures of these novel compounds were determined exactly using mass spectrometry and NMR.

This result clearly demonstrates that the combination of in silico analysis of secondary metabolite gene clusters, the application of molecular genetic tools, and precursor-directed biosynthesis can deliver novel derivatives, which cannot be obtained easily by chemical methods. This technology is transferable to the synthesis of other important drugs containing nonproteinogenic amino acids, like vancomycin, as was demonstrated recently when the technique was used to generate the first fluorinated vancomycin-like derivative [13]. Furthermore, precursor-directed biosynthesis offers the opportunity to study the substrate specificity of enzymes including NRPS, oxygenases, halogenases, and others that are involved in modification of the natural products. Such information will provide us with information on the possible use of these enzymes in further combinatorial approaches.

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Selected Reading

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