Methods in ENZYMOLOGY

Volume 459

Complex Enzymes in Microbial Natural Product Biosynthesis, Part B: Polyketides, Aminocoumarins and Carbohydrates

Edited by

David A. Hopwood

VOLUME FOUR HUNDRED AND FIFTY-NINE

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PREFACE

The complex structures of microbial natural products have fascinated chemists for decades. As the tools of chemistry and biochemistry were sharpened, huge advances in understanding natural product biosynthesis were made, but there were still barriers to a satisfactory understanding. Many such impediments were due to the instability of intermediates in the biosynthetic pathways, which hampered chemical analysis. At the same time, a frequent inability to obtain active cell–free preparations severely limited the success of biochemical approaches. A striking example of these limitations is provided by the polyketides, the largest and most important family of secondary metabolites. Chemistry and biochemistry had deduced the relationships between polyketide and fatty acid biosynthesis and had revealed the basic biochemical reactions involved, but there was little understanding of the "programming" of the enzymes, that is control of the variables that make the polyketides such a varied class of chemicals: choice of starter and extender units for carbon chain building, and control of chain length, degree of reduction of keto groups, and chirality of carbon and hydroxyl branches. Isolation of the actinomycete gene clusters that encode the polyketide synthases, their sequencing, and their manipulation into unnatural combinations in the early 1990s changed the landscape almost overnight. There followed a period in which genetics provided the primary stimulus to much of the research in natural product biosynthesis. Now, chemistry, genetics, enzymology, and structural studies are working synergistically to reveal the details of biochemical control.

This two-volume set of *Methods in Enzymology* reflects these developments in the study of natural product biosynthesis. As expressed by Mel Simon in his invitation to edit the set, it is especially timely in view of the increasing need for novel bioactive natural products, especially antibiotics and anticancer drugs, and the new possibilities for addressing this need by carrying out "chemistry through genetics" and by studying the gamut of potential natural products revealed by the sequencing of microbial genomes.

We begin Volume A with the isolation and screening of various kinds of microorganisms, to provide the raw material for subsequent fundamental studies or for the development of natural products as drugs. Then come three chapters dealing with the regulation of secondary metabolite production in actinomycetes—the group of filamentous soil bacteria that are preeminent secondary metabolite producers—and how an understanding of such regulation can furnish compounds that would otherwise be

hard to obtain. Next are chapters covering the cloning and analysis of biosynthetic pathway genes and computer-based methods for predicting the products encoded by gene sets for two key classes of secondary metabolites, the polyketides and nonribosomal peptides, from DNA sequence data, as well as articles describing innovative approaches to probing their biosynthesis. Two final chapters in the first section deal with the biosynthesis of sugars and their attachment to secondary metabolite aglycones, thereby conferring biological activity.

The section on peptide natural products begins with an overview of nonribosomal peptide biosynthesis, followed by a detailed description of methods for studying the biosynthesis of the amino acids and other precursors that function as building blocks in their assembly, as well as a chapter on the heterologous expression of nonribosomal peptide synthetase genes. Next come chapters on a specific class of compounds in this superfamily, the cephem beta-lactams, on a special type of iron-chelating siderophore, and on the important glycopeptide and lipopeptide families of antibiotics. Moving to ribosomally synthesized peptide natural products, two chapters cover the lantibiotics, a topic of increasing current focus in the search for antibiotics effective against resistant pathogens. We end Volume B with another example of ribosomally synthesised peptides, this time coupled with techniques for metagenomics mining.

Volume B is dominated by the polyketides, reflecting their preeminence as natural products. Kira Weissman introduces polyketide synthesis and the different types of polyketide synthases, and puts the 16 chapters in this section elegantly into context, making redundant any further remarks here, except to note the absence of a chapter on the type III polyketide synthases, an omission stemming from the last-minute withdrawal of the author chosen for this topic. The section on aminocoumarins contains a single chapter that provides a particularly fine example of the application of molecular genetics to another class of compounds, with considerable potential for the generation of "unnatural natural products" by genetic engineering techniques first developed for the polyketides. The volume ends with a section on carbohydrate-type natural products, with two chapters on aminoglycosides and one on the biosynthesis of the TDP-deoxysugars that play such a crucial role in conferring biological activity on a whole range of secondary metabolites, harking back to the chapters on sugar biosynthesis in Volume A.

Inevitably, the choice of topics to include in these volumes is somewhat arbitrary. The peptides and polyketides chose themselves because of their importance among natural products, especially as antibiotics, and because of the huge amount of recent research devoted to them. Historically, the aminoglycosides were centre-stage in the early days of antibiotic discovery—streptomycin was the first important actinomycete antibiotic to be described, and only the second, after penicillin, from any source to be a

medical marvel—and they probably still make up the third largest chemical family of antibiotics, earning them a place in Volume B. Several other classes of microbial natural products were contenders for inclusion: aminocoumarins, terpenoids, and tetrapyrroles, among others. However, space constraints precluded inclusion of all of them, and in the end only the aminocoumarins made the cut. Hopefully, other classes will take their place in a further volume in due course, along with fuller coverage of natural product production by a wider range of microorganisms outside of the actinomycetes.

I am most grateful for the enthusiastic response that greeted my invitations to contribute to this project. Inevitably, leaders in the field have many calls on their time, but it was most gratifying that nearly all my invitees either accepted or offered suggestions for alternative authors. I am especially grateful to Greg Challis, Chaitan Khosla, Tom Simpson, and Chris Walsh for their insightful ideas. To those who accepted—as well as to the many co-authors who were recruited to the writing—thank you for the time and effort that went into the preparation of the chapters and to the friendly way in which you all responded to my—usually minor—editorial suggestions, making my task a very pleasant one.

David A. Hopwood

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