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Natural product biosynthesis — a Renaissance

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In the last decade, we have witnessed remarkable progress in finding new pathways and novel reactions in natural product biosynthesis. The rapid growth of genomic and metagenomic sequencing data combined with technical advances in genetic as well as synthetic biology have played a significant role in the recent achievements made in this field. Cloning and heterologous expression of these biosynthetic genes has facilitated the identification and characterization of new enzyme chemistries shedding considerable light on the biochemical origin of complex natural products. In terms of basic science, the mechanistic investigation of these recently discovered and unusual transformations has enhanced our fundamental understanding of enzyme catalysis. From a more practical perspective, this information is in turn offering new insights into how these biosynthetic machineries may be engineered and exploited for drug discovery and production. In this issue, some of these advances are highlighted in 13 expert reviews.

Tropolones are seven-membered aromatic rings originating from the ring expansion of alkylated phenyl ring-containing intermediates derived from polyketide, alkaloid, terpene and shikimate biosynthetic pathways. In the contribution of Cox and Al-Fahad, the proposed pathways for the construction of tropolones in bacteria, plants and fungi are discussed. The key reaction appears to proceed via enzyme catalyzed hydroxylation of a phenyl ring followed by isomerization to the expanded product via either a pinacol-type rearrangement or a hydroxycyclopropane intermediate. A number of instances are considered including thiotropocin, tropodithietic acid, colchicine and β -thujaplicin biosynthesis. The mechanism of the non-heme mononuclear iron-dependent dioxygenase, TropC, from the stipitatic acid biosynthetic pathway is also discussed.

Echinomycin is a potent antitumor compound owing to its ability to bis-intercalate DNA. The review by Watanabe and coworkers provides an update on how this complex natural product is biosynthesized. Importantly, both the flavoenzyme catalyzing adisulfide bond formation, Ecm17, and the thioesterase, Ecm7, show relaxed substrate specificity emphasizing their potential value in pathway re-engineering to generate diversified analogues. Furthermore, the disulfide product of Ecm17 appears to be further processed via the formation of a reactive sulfonium ylide intermediate after enzyme catalyzed *S*-methylation. The use of methylation to generate reactive cationic intermediates so as to

facilitate further structural modification appears to be a motif of catalysis, that is, only just beginning to be appreciated.

Current progress in the field of cyclotide biosynthesis is summarized by Craik and Malik. The stability of these disulfide-rich macrocyclic peptides arises from their cyclic structure and unique knotted topology along with the presence of three conserved disulfide linkages. It is suggested that disulfide bond formation occurs first to lock the conformation of cyclotide precursors. This leaves the *N*-termini and *C*-termini in close proximity to facilitate the cyclization, which may take place via a transpeptidation reaction involving an acyl-enzyme intermediate.

The biosynthesis of lasalocid, an ionophore polyether-type natural product, is the focus of the review by Oikawa and coworkers. Recent studies have shown that stereospecific epoxidation of the PKS-bound diene precursor is catalyzed by an FAD-dependent monooxygenase, Lsd18, and that opening of the epoxide is catalyzed by a hydrolase, Lsd19. Structural and mutagenesis studies on Lsd19 have demonstrated that the mechanism of epoxide opening involves acid/base catalysis, and that both the *N*-terminal and *C*-terminal domains of Lsd19 independently catalyze single cyclization reaction during lasalocid biosynthesis. The second cyclization proceeds in 6-*endo*-mode instead of the energetically favored 5-*exo*-mode may be ascribed to active site pre-organization in Lsd19.

The review from Metsä-Ketelä and coworkers summarizes the current knowledge regarding the biosynthesis of pyranonaphthoquinones such as alnumycin. A type II polyketide synthase along with a host of tailoring enzymes have been well characterized in the assembly of the polycyclic aromatic core of alnumycin. However, the final product also possesses adioxane ring, the introduction of which appears to involve novel chemistry. The dioxane is derived from D-ribose-5-phosphate and is incorporated in four distinct steps including a peculiar multistep bond cleavage and rearrangement catalyzed by the novel cofactor-independent oxidase, Aln6.

The review from Liu and coworkers provides a brief account of recent advances in understanding the biosynthesis of isoprenoid precursors. While the mevalonic acid pathway has been studied for decades, research on the more recently discovered methylerythritol phosphate (MEP) pathway is still in its infancy. Specifically highlighted in this review are the current mechanistic models for two key enzymes, IspG and IspH, in the MEP pathway. A detailed mechanistic understanding of these enzymes is expected to be important for future pharmaceutical and biomedical applications.

Peck and van der Donk review progress in elucidating the biosynthesis of phosphonate and phosphinate natural products. A number of unusual reactions are discussed, including cleavage of the C–C bond in 2-hydroxyethylphosphonate (2-HEP) by two non-heme mononuclear iron-dependent enzymes, 2-HEP dioxygenase and methylphosphonate synthase. Also highlighted is the epoxide ring formation catalyzed by a closely related enzyme, (*S*)-hydroxypropylphosphonate epoxidase in fosfomycin biosynthesis. Catalysis by HppE involves novel oxidative chemistry and has been the focus of much recent mechanistic scrutiny.

Current knowledge regarding enzymes capable of decomposing phosphonate-containing compounds is also summarized in this issue by Kamat and Raushel. The C–P bond is chemically stable, and metabolic enzymes have evolved in bacteria to liberate phosphate from various phosphonate compounds used as nutrient sources. The PhnZ-catalyzed C–P bond cleavage of 2-amino-1-hydroxyethylphosphonate is one intriguing example that may involve a high-valent Fe^{III}–Fe^{IV}-oxo species reminiscent of *myo*-inositol oxygenase. Also considered is the radical *S*-adenosyl-L-methionine (SAM) enzyme, PhnJ, which has been proposed to utilize a protein thiyl radical to facilitate homolytic cleavage of a C–P bond.

As exemplified by PhnJ, many recently discovered novel transformations are catalyzed by members of the radical SAM superfamily of enzymes. The reductive homolysis of SAM using a [4Fe–4S]¹⁺ cluster to produce a 5′-deoxyadenosyl radical (5′-dAd•) is the distinguishing feature of these enzymes, and their enzymology remains a burgeoning area of study. Investigations into how these enzymes generate and control the reactive radical intermediates in their catalytic cycles continue to offer new insights and surprises with regard to enzyme chemistry. In addition to PhnJ, a number of other newly identified radical SAM enzymes are highlighted in this issue. Together, they attest to the wide-ranging catalytic capabilities and great mechanistic diversity of this superfamily of enzymes.

In the review by Fujimori, an outline of radical SAM-mediated methylation reactions is presented. RlmN and Cfr catalyze a unique methylation reaction by inserting a methylene unit, instead of an intact methyl group, into an adenosyl moiety of the 23S rRNA. This reaction requires two equivalents of SAM: one acts as a carbon donor and the other as a radical initiator and oxidant. In contrast, the methylation of tryptophan in thiostrepton biosynthesis follows a different course and involves a cobalamin-dependent radical SAM enzyme, TsrM. While SAM represents the methyl donor to generate methylcobalamin, no reductive cleavage of SAM has been observed. Thus, the mechanism of TsrM may be unique among the radical SAM enzymes.

The involvement of radical SAM enzymes in the biosynthesis of thioether functionalities is discussed in the review by Flühe and Marahiel. These enzymes appear to utilize a second auxiliary [4Fe–4S] to both coordinate the thiol donor and serve as a one-electron acceptor during radical-mediated generation of the thioether product. AlbA from the subtilisin A pathway and SkfB from the sporulation killing factor pathway are highlighted as examples of this new and intriguing mode of biological thioether formation.

TYW1 is another radical SAM enzyme required for the biosynthesis of the tRNA base wybutosine and is the focus of the review by Young and Bandarian. In a reaction, that is, still not completely understood, TYW1 couples the C2–C3 fragment of pyruvate to *N*-methylguanosine to yield imG-14. The enzyme utilizes two [4Fe–4S] clusters, only one of which is required for 5′-dAd• generation. While the role of the second auxiliary cluster remains unclear, it does appear to be important for a homolytic C–C bond cleavage reaction during the catalytic cycle. The overall reaction of TYW1 is unprecedented among the radical SAM enzymes and one well worth attention.

The identification of the 22nd genetically encoded amino acid, pyrrolysine, is an important discovery in recent biology. In contrast to the large number of posttranslationally modified amino acids found in proteins, pyrrolysine is pretranslationally derived from two molecules of lysine as described in the review by Krzycki. PylB is a radical SAM enzyme responsible for the key step in this process whereby a methylated ornithine is produced from lysine as the first intermediate. The PylB-catalyzed intramolecular rearrangement is analogous to that of B₁₂-dependent glutamate mutase, but is currently the only known example of such among the radical SAM enzymes.

The review by Wen Liu and coworkers summarizes the biosynthesis of nosiheptide (NOS), a thiopeptide antibiotic. The NOS ribosomal precursor peptide is processed by a series of post-translational modifications including cyclodehydration/dehydrogenation, dehydration, and a [4 + 2] cycloaddition. NOS also demonstrates an indolic acid (IA) moiety derived from a tryptophan residue under the action of two radical SAM enzymes, NosL and NosN. NosN is responsible for a radical induced methylation reaction, whereas NosL is proposed to catalyze both removal of the C_α-N unit and migration of the carboxylate to the indole ring. The proposed mechanism for NosL is an unprecedented radical induced fragmentation-recombination, and should offer considerable insight into the biological production of highly modified tryptophan residues.

As these examples indicate, we are only just beginning to understand the exquisite capabilities of enzymes to catalyze complex reactions. This is particularly clear for the radical SAM enzymes, which by virtue of their signature motif has permitted more than 45 000 proteins to be identified as potential members of this enzyme superfamily on the basis of sequence analysis alone. However, only a small fraction of the predicted radical SAM enzymes have been examined, and it can only be imagined how many unprecedented and exciting new chemistries wait to be discovered within the greater context of natural product biosynthesis.

Biographies

Hung-wen (Ben) Liu graduated from Tunghai University (Taiwan), received a Ph.D. in Chemistry from Columbia University, and did his post-doctoral research at MIT. In 1984, he joined the faculty at the University of Minnesota, where he was promoted to Full Professor in Chemistry in 1994. In 2000, he moved to the University of Texas, Austin, where he is now the George H. Hitchings Regents Chair in Drug Design and Professor of Medicinal Chemistry, Chemistry, and Biochemistry.

Tadhg Begley obtained his B.Sc. from The National University of Ireland in 1977 and his Ph.D. from the California Institute of Technology (P. Dervan) in 1982. He carried out postdoctoral studies at the University of Geneva (W. Oppolzer) and at MIT (C. Walsh). After 23 years in the Cornell Chemistry Department, he moved to Texas A&M University in 2009 where he is the D. H. R. Barton and Robert A. Welch Chair in Chemistry. Begley's research is focused on the mechanistic enzymology of complex organic transformations, particularly those found on the vitamin biosynthetic pathways. Dr Begley served as the Chief Advisor for the "Wiley Encyclopedia of Chemical Biology". He edited a volume of "Comprehensive

Natural Products Chemistry” on Cofactors and coauthored “The Organic Chemistry of Biological Pathways” with John McMurry.

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