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Previews

Modular NRPSs Are Monomeric

NRPSs, PKSs, and hybrid NRPS/PKSs are modular proteins with similar assembly-line organizations. Although PKSs function as dimers, new data demonstrate that functional NRPSs are monomeric. This discovery has significant implications for engineering artificial assemblies for the production of novel biotherapeutics.

In bacteria and fungi, a broad range of secondary metabolites are elaborated by two families of large, multimodular proteins that function in an assembly-line manner, the NRPSs (nonribosomal peptide synthases) and the PKSs (polyketide synthases). The products of these modular enzyme systems include many important pharmaceuticals, such as penicillin, vancomycin, and cyclosporin (NRPS), and erythromycin and avermectin (PKS). The NRPSs and PKSs are organized according to a common modular assembly-line theme in which each module contains a domain for substrate selection, transfer, and condensation, and the reaction intermediates are tethered to the complex through covalent linkage to a phosphopantetheine thiol moiety that forms part of a peptidyl, or acyl, carrier protein domain [1]. The prototypical NRPS and PKS modules consist of three functional domains. In the NRPSs, they are an adenylation domain, responsible for amino acid selection and activation to an aminoacyl-AMP, a peptidyl carrier protein domain, and a condensation domain, which is responsible for formation of the C-N amide bond with the aminoacyl moiety attached to the peptidyl carrier protein domain of the adjacent module. In the PKSs, the three basic domains are an acyltransferase domain, which loads the substrate, typically a malonyl or methylmalonyl moiety, an acyl carrier protein domain, and a β-ketoacyl synthase domain, which catalyzes the condensation reaction and the formation of new C-C bonds. In both types of modular system, the constituent domains are linked sequentially in the order condensing domain, substrate selection domain, peptidyl/acyl carrier protein. The structural diversity of the products of these assembly lines can be increased through the inclusion of additional domains for epimerization, N-methylation, or cyclization in the NRPSs, and additional domains for β-ketoacyl reduction, dehydration, and enoylreduction in the PKSs. In both systems, chain initiation is catalyzed by the most upstream module, the intermediates are passed successively to the adjacent module in the assembly line, and chain termination is effected in the most downstream module by a thioesterase domain that releases the fully formed peptide, or polyketide, from its covalent linkage to the ultimate phosphopantetheine moiety. Thus, the ordering of the individual modular units in the assembly line determines the amino acid sequence of the peptide or the final structure of the polyketide [2, 3]. Remarkably, in some organisms mixed assembly lines containing both NRPS and PKS modules catalyze the formation of complex secondary metabolites, such as the anticancer agents bleomycin and epothilone and the toxins microcystin and yersiniabactin [1]. In recent years, these modular proteins have become attractive targets for genetic engineering and manipulation in order to produce novel compounds with useful therapeutic properties.

A substantial body of experimental evidence indicates clearly that the modular PKSs, like the structurally and functionally related animal fatty acid synthases, are homodimeric proteins in which some of the reaction steps are catalyzed at the subunit interface [4, 5]. Thus, the modular PKSs are "dual-track" assembly lines capable of the production of two identical products side by side. In contrast, structural analyses of free-standing condensation, adenylation, peptidyl carrier protein, and thioesterase domains derived from NRPSs indicate that they are all monomeric, suggesting that the entire NRPS assembly lines may be constituted from "single-track" monomeric modules [6–10]. Clearly, elucidation of the quaternary structure of the modular NRPSs is important for understanding their functional properties and is likely to be critical for the successful engineering of novel NRPSs and hybrid NRPS/PKS systems.

In the study reported in this issue of *Chemistry & Biology*, Sieber et al. have painstakingly evaluated the oligomeric state of several NRPSs, employing the same range of physical, chemical, and biochemical approaches that were used to establish the dimeric nature of the PKSs and animal fatty acid synthases [11]. The results of gel filtration, equilibrium ultracentrifugation, and chemical crosslinking studies indicate clearly that, except at relatively high protein concentrations, intact NRPS modules are monomeric. Furthermore, several biochemical experiments designed to detect putative heterodimers formed from differentially tagged or mutated parental NRPSs all gave negative results. Although the inability to detect heterodimers could possibly have resulted from technical difficulties in establishing optimum experimental conditions for their formation, the results are entirely consistent with the findings of the biophysical and crosslinking studies. Collectively then, the results of this new study, together with the earlier structural data indicating that free-standing domains of NRPS are monomeric, provide compelling evidence indicating that the entire modular structures are indeed functional as monomers.

What of the NRPS/PKS hybrid systems? How are dual-track PKS assembly lines coupled to single-track NRPSs? The authors report that HMWP1, a subunit of the yersiniabactin synthetase, which consists of a five-domain PKS module fused to a four-domain NRPS module, can in fact form dimers. Thus, it is possible that homodimeric interactions within PKS modules may be

sufficient to stabilize an overall dimeric structure for the hybrid proteins, despite the absence of interactions between equivalent NRPS modules. The authors envision a structure in which a dual-track assembly line is preserved in these hybrid proteins by the protrusion of pairs of monomeric NRPS loops from a dimeric PKS core, in much the same way as has been proposed for the optional β-ketoacyl reduction, dehydration, and enoylreduction domains of PKS modules themselves [4]. However, NRPS and PKS modules are not always connected covalently in hybrid assembly lines. For example, the hybrid NRPS/PKS proteins responsible for the biosynthesis of myxothiazol, epithilone, and yersiniabactin contain NRPS modules that appear to function in *trans* with adjacent PKS modules on separate polypeptides [1]. In the case of exclusively PKS modular proteins, it has been proposed that fidelity in the intermodular transfer of intermediates to the appropriate downstream polypeptide depends both on the specificity of the interaction between the acyl carrier protein domain of the upstream (donor) polypeptide and the downstream (acceptor) β-ketoacyl synthase domain and on the presence of complementary linker regions at the N and C termini of the interacting polypeptides [12]. Whether similar mechanisms could be operative in facilitating interactions between NRPS and PKS modules located on separate polypeptides and in the maintenance of a dual-track assembly line through these junctions remains to be determined.

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Toward Bioengineering Anticancer Drugs

The biosynthetic route for enediyne production remained mysterious until two independent groups recently reported the genes that orchestrate enediyne synthesis in two different microorganisms. These discoveries lay the foundations for engineering this pathway to generate improved anticancer drugs.

The enediyne class of antitumor agents are complex natural products whose unique structure, mechanism of action, and potent cytotoxicity have earned the interest of chemist and biologists. The total synthesis of members of the class has been achieved [1], and elegant research based upon fundamental work done by Robert Bergman in the 1970s revealed the way in which enediynes cleave DNA [2]. Recently, a new anticancer drug consisting of one member of the class conjugated to a cancer cell-recognizing antibody has been approved for use in acute myelogenous leukemia patients [3]. Despite these advances, a fundamental question concerning the biosynthetic origin of this group of highly unsaturated natural products remained unresolved. Now, two groups working with two different enediyne-producing organisms have reported the biosynthetic genes responsible for the construction of these natural products. Their results indicate that the enediynes share a common polyketide biosynthetic pathway. This work lays the foundation for bioengineering this pathway in order to produce improved anticancer drugs.

The enediynes contain at their core two acetylenic groups flanking a double bond or incipient double bond within a nine- or ten-membered ring chromophore. The key to the extreme cytotoxicity of this group of natural products is the ability of this unstaturated core to undergo a so-called Bergman cyclization to produce a reactive diradical intermediate. The interaction of the diradical with double-stranded DNA results in the oxidative cleavage of the DNA and ultimately cell death. Most of the nine-membered ring enediynes are produced as complexes with specific proteins that serve to stabilize the chromophore, whereas the more stable ten-membered enediynes are found free from stabilizing proteins. Biosynthetic studies of both nine- and ten-membered enediynes have been carried out by feeding specifically labeled biosynthetic building blocks, such as acetate, to enediyne-producing organisms [4]. These labeling experiments demonstrated that both nine- and ten-membered enediyne chromophores are constructed of head-