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Engineered polyketides: Synergy between protein and host level engineering

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ABSTRACT

Metabolic engineering efforts toward rewiring metabolism of cells to produce new compounds often require the utilization of non-native enzymatic machinery that is capable of producing a broad range of chemical functionalities. Polyketides encompass one of the largest classes of chemically diverse natural products. With thousands of known polyketides, modular polyketide synthases (PKSs) share a particularly attractive biosynthetic logic for generating chemical diversity. The engineering of modular PKSs could open access to the deliberate production of both existing and novel compounds. In this review, we discuss PKS engineering efforts applied at both the protein and cellular level for the generation of a diverse range of chemical structures, and we examine future applications of PKSs in the production of medicines, fuels and other industrially relevant chemicals.

Contents

Abbreviations: PK, Polyketide; PKS, Polyketide synthase; FAS, Fatty acid synthases; KS, Ketosynthase; AT, Acyltransferase; ACP, Acyl carrier protein; DH, Dehydratase; KR, Ketoreductase; ER, Enoylreductase; TE, Thioesterase; LM, Loading module; CoL, CoA-Ligase; R, Reductase domain; PDB, Precursor directed biosynthesis; TKL, Triketide lactone; DE, Dimerization element; SNAC, N-acetylcysteamine; DEBS, 6-deoxyerythronolide B synthase; SARP, *Streptomyces* antibiotic regulatory protein; LTTR, LysR-type transcriptional regulator; PCC, Propionyl-CoA carboxylase.

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1. Introduction

Polyketides are one of the largest classes of natural products, possessing immense structural diversity and complex chemical architectures. Many polyketides (PKs) are among the most important secondary metabolites for their applications in medicine, agriculture, and industry. Examples include anticancer drugs (epothilone) [1,2], antibiotics (erythromycin) [3], insecticides (spinosyn A) [4] and antifungals (amphotericin B) [5]. These particular examples of polyketides are biosynthesized by multimodular enzyme complexes known as type I modular polyketide synthases (PKSs). Working in an assembly-line fashion, multimodular PKSs assemble and tailor readily available acyl-CoAs within the host cell into large, complex, chiral molecules [6]. Each of these PKSs comprises a series of modules that can be further dissected into a series of domains responsible for the extension of the polyketide backbone through condensation and selective reductive processing of an acyl-CoA building block. The collinear architecture of these modules, apparent by inspection of the domains present and the predictive selectivity motifs harbored within, provide insights into the chemical connectivity and stereochemical configuration of the polyketide metabolite from analysis of its coding sequence.

One of the best-studied PKSs is the 6-deoxyerythronolide synthase (DEBS) (Fig. 1A), which is responsible for synthesizing the macrolactone core of the antibiotic, erythromycin [7]. The catalytic domains of DEBS are expressed within modules that are each responsible for a single round of chain elongation and reductive processing. To this end, the loading acyltransferase (AT) domain loads the acyl carrier protein (ACP) with a starter unit derived from propionyl-CoA (Fig. 1A). The ketosynthase (KS) within each module catalyzes decarboxylative carbon-carbon bond formation between an acyl precursor and the ACP-bound methylmalonyl derivative. Unlike fatty acid synthases (FASs), the occurrence of reductive domains within modules varies, and PKS intermediates typically exhibit various levels of reduction. If present, the ketoreductase (KR) converts the β-ketone to an alcohol using NADPH. The dehydratase (DH) eliminates the alcohol to form an olefin, and the enoylreductase (ER) utilizes NADPH to reduce the olefin to a methylene. Finally, a thioesterase (TE) domain, located at the terminal of DEBS 3 module, catalyzes the release and cyclization to produce the macrolactone, 6-deoxyerythronolide (6-dEB). The structure and mechanism of each PKS domain is reviewed in detail elsewhere [8–12].

With this collinear biosynthetic logic in mind, engineered PKSs have the potential to become an effective retrobiosynthetic platform to produce molecules that are difficult or too complex to acquire via traditional synthetic means (Fig. 1B–C). From DNA sequence, one could control chemical structure by successfully modifying and rearranging existing polyketide modules and domains [13,14]. Moreover, rationally-designed PKSs could be introduced into a variety of engineered hosts [15–17] capable of expressing these large PKS complexes while providing the necessary precursor metabolites to biosynthesize a target chemical. In this review, we highlight PKS engineering efforts at both the protein level and the host/cellular level. We further aim to describe PKS engineering efforts within the context of metabolic engineering, and introduce the idea of successful PKS/host modifications for both traditional medicinal applications as well as the production of fuels and commodity chemicals.

2. PKS protein engineering

The ability to tailor the molecular architecture of polyketide metabolites through the inclusion of various reductive domains and/or domains with altered selectivity has long been the promise of PKSs as a retrobiosynthetic platform. In this section, we discuss the current knowledge of PKS engineering at the protein level. We have divided the PKS protein engineering section into sections based on domain type. Within the types of domains, we have selected the most engineerable targets. We will not focus on KS or ACP domain engineering in this review, as they are arguably the least targetable domains based on the chemistry and functions they perform, respectively. In addition, methyltransferase domains, which transfer an S-adenosyl-methionine-derived methyl group to the α-carbon of the β-keto intermediate, are somewhat rare and less well characterized, and thus will not be discussed here. In each section, we will first give a basic overview of the current state of knowledge regarding the specific domain(s) in question. Next, we will highlight some significant accomplishments in engineering, both via site-directed mutagenesis and/or domain swapping experiments. Because of the extensive amount of published PKS research, we cannot include all examples of PKS engineering within the scope of this review. Nevertheless, numerous representative examples are highlighted.

2.1. Loading modules

Nature has evolved several mechanisms for activating acyl substrates to initiate PK biosynthesis. To begin chain formation, modular type I PKSs employ a loading module (LM) to select the priming unit. LMs are categorized based on their domain architecture and the mechanism by which each activates substrates to begin chain formation. Although LMs are not officially characterized within the field, for simplicity within this review we will refer to each class of LMs with a representative letter (e.g. “type A” or “A-type”) so as not to confuse the reader with the type I, II, or III PKS designations used to describe the entire assembly line systems.

The most common LM organization consists of a condensation-incompetent KS^{Q} (named for the active site C→ Q mutation), AT, and ACP domain (Type A LM) [18,19]. In type A LMs, the KS^{Q} decarboxylates malonyl- or methylmalonyl-CoA to yield acetyl- or propionyl starter units, respectively (Figs. 2 and 3) [20]. The AT domains of these modules are strictly specific for CoA esters of dicarboxylic acids (malonyl- or methylmalonyl-CoA) [20] and share

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