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Review

Non-modular polyketide synthases in myxobacteria

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ABSTRACT

Myxobacteria are prolific producers of a wide variety of secondary metabolites. The vast majority of these compounds are complex polyketides which are biosynthesised by multimodular polyketide synthases (PKSs). In contrast, few myxobacterial metabolites isolated to date are derived from non-modular PKSs, in particular type III PKSs. This review reports our progress on the characterisation of type III PKSs in myxobacteria. We also summarize current knowledge on bacterial type III PKSs, with a special focus on the evolutionary relationship between plant and bacterial enzymes. The biosynthesis of a quinoline alkaloid in *Stigmatella aurantiaca* by a non-modular PKS is also discussed.

Contents

1. Introduction

Myxobacteria are soil-dwelling, Gram-negative bacteria which are notable not only for their multi-cellular 'social' lifestyles (Gerth et al., 2003), but for biosynthesis of structurally diverse natural products with potential as pharmaceuticals. These metabolites principally comprise polyketides and non-ribosomal peptides, but also include volatiles as well as plant-like steroids and alkaloids (Weissman and Müller, 2009). Polyketides are constructed from a common set of simple precursors (small acyl-CoA thioesters, such as malonyl-CoA and methylmalonyl-CoA), but the assembly process occurs by three distinct mechanisms. The 'reduced' or 'complex' class of polyketides are biosynthesised by gigantic multifunctional enzymes, termed type I or modular polyketide synthases (PKSs). In these PKSs, catalytic domains are covalently linked and grouped into modules, such that each module carries out one round of chain extension. In contrast, the type II and type III PKSs both give rise to aromatic polyketides. Type II PKSs are composed of a complex of discrete, iteratively-acting enzymes (minimally two ketosynthase-like condensing enzymes KSα and KSβ, the first of which contributes the condensation active site, and an acyl carrier protein (ACP)), whereas type III PKSs are small, dimeric proteins which accomplish all reactions required for polyketide synthesis within a single active site. The majority of myxobacterial biosynthetic pathways incorporate type I PKSs, while type II and type III systems are relatively rare.

One possible explanation for the relative dearth of aromatic polyketides in myxobacteria is that the encoding pathways are silent under standard growth conditions, and so the associated metabolites have not yet been isolated. Alternatively, type II and III PKSs may be rare in Gram-negative bacteria (Sandmann et al., 2007; Brachmann et al., 2007). We have addressed this question directly for the type III PKS by 'mining' the genome data of three myxobacterial strains which include *Myxococcus xanthus* DK1622 (Goldman et al., 2006), *Sorangium cellulosum* So ce56 (Schneiker

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et al., 2007) and *Stigmatella aurantiaca* (Ronning and Nierman, 2007) for putative type III PKS genes, as well as by heterologous expression and assays with purified proteins *in vitro*.

Our analysis has revealed several type III PKSs in myxobacteria, albeit at reduced numbers relative to type I systems. In this study, we compare these machineries to known bacterial PKSs, and also assess the evolutionary relationship between bacterial and plant enzymes. Finally, we discuss the biosynthesis of the quinoline alkaloid aurachin, which was initially thought to arise from a type III PKS, but which is now known to be generated by an unusual non-modular PKS.

2. Bacterial type III PKSs catalyse diverse reactions

Although they are the simplest of the three PKS types, the type III PKSs are remarkable catalysts. These enzymes exploit a single active site to iteratively perform a complex series of reactions: loading of an acyl-CoA thioester to the active site cysteine, chain extension via decarboxylative condensation of an extender unit (usually malonyl-CoA), and intramolecular cyclisation to release the final product. By varying the choice of starter unit, the number of condensation steps, and the mechanism of cyclisation (aldol condensation, Claisen condensation, or lactonisation), type III PKSs produce of a wide array of different metabolites (Austin and Noel, 2003).

The founding member of the type III PKS family, chalcone synthase (CHS), was first isolated from plants, where it is ubiquitous (Austin and Noel, 2003). CHS enzymes catalyse the condensation of three molecules of malonyl-CoA to a *p*-coumaroyl-CoA starter which is derived from the phenylpropanoid pathway. Subsequent Claisen-type cyclisation of the tetraketide leads to the formation of chalcone (Fig. 1). Since the discovery of CHS, many variants of CHS-like proteins with divergent functions have been identified; some of them, such as acridone synthase, catalyse non-CHS reactions (Fig. 1). A further surprise revealed by the rapid expansion in genome sequence data, is that CHS-like proteins are also widespread in fungi and bacteria. However, bacterial type III PKSs generally share weak overall sequence homology to plant CHSs (typically 25% sequence identity), indicating they may perform reactions different from those of classical CHSs.

On the basis of the structures of their products, five classes of bacterial type III PKSs have been characterised to date (Fig. 1). Three of these enzymes use malonyl-CoA both as starter and extender units, but differ in the number of monomers used in chain extension and the pattern of cyclisation. For example, 1,3,6,8-tetrahydroxynaphthalene (THN) synthase (referred to as RppA) from *Streptomyces griseus* (Funa et al., 1999), the first bacterial type III PKS to be discovered, assembles a chain from five molecules of malonyl-CoA, and then catalyses two successive cyclisation steps to give THN; oxidation to the final product flaviolin is spontaneous. The RppA homologue from *Streptomyces coelicolor* A(3) 2 has been structurally and kinetically characterised, providing detailed insight into its mechanism (Izumikawa et al., 2003; Austin et al., 2004). The type III PKS PhlD from *Pseudomonas fluorescens* (Achkar et al., 2005) is representative of the second class, which constructs a phloroglucinol product from three units of malonyl-CoA. A third class of type III PKS, exemplified by DpgA from *Amycolatopsis orientalis*, assembles dihydroxyphenylglycine (DHPG) from four malonate monomers (Li et al., 2001).

The final two classes of type III PKSs use long chain acyl-CoAs rather than malonyl-CoA as starter units. These comprise: (1) alkylresorcinol synthases, which are exemplified by ArsB from *Azotobacter vinelandii* (Funa et al., 2006; Miyanaga et al., 2008) and *Streptomyces* resorcinol synthase A (SrsA) from *S. griseus* (Funabashi et al., 2008); and (2) alkylpyrone synthases, which include Gcs (SCO7221) from *S. coelicolor* (Song et al., 2006) that is required for the production of the pyrone metabolite germicidin, and ArsC from *A. vinelandii* (Funa et al., 2006; Miyanaga et al., 2008). Both ArsB and ArsC from *A. vinelandii* are responsible for phenolic lipids biosynthesis during cyst formation (encystment). Moreover, several cryptic bacterial type III PKSs have been shown to accept long chain acyl-CoAs to generate α-pyrones *in vitro*. Such enzymes include Pks11 and Pks18 from *Mycobacterium tuberculosis* (Saxena et al., 2003), as well as SCO7661 from *S. coelicolor* (Grüschow et al., 2007). However, as pyrones commonly arise from derailment of normal biosynthesis, the true functions of these type III PKSs remain unknown.

Studies of bacterial type III PKS have revealed further features which differentiate them from plant CHS enzymes. While classical CHSs employ starter units activated as CoA thioesters, some bacterial type III PKSs use starter units bound to ACPs. For example, the C22–C26 acyl chain starter units used in phenolic lipid biosynthesis, are transferred directly from the ACP domains of the type I fatty acid synthase (FAS) to the type III PKS ArsB and ArsC (Miyanaga et al., 2008). Furthermore, Grüschow et al. (2007) demonstrated that SCO7661 from *S. coelicolor* uses both CoA- and ACP-tethered starters (Grüschow et al., 2007), although the physiological relevance of this finding is not yet known. Moreover, a fatty acid metabolite, 3-oxo-4-methyl-pentyl-ACP, has been proposed to be the priming unit for SCO7221 during germicidin biosynthesis (Song et al., 2006). Taken together, these findings suggest that in contrast to plant CHS systems, accepting acyl starters from ACPs is not uncommon for bacterial type III PKSs.

The second novel feature is the ability of bacterial type III PKSs to employ acyl-CoAs other than malonyl-CoA as extender units. For example, feeding studies have suggested that SCO7221 uses ethylmalonyl-CoA as extender unit (Fig. 1) (Song et al., 2006), while SrsA from *S. griseus* is able to catalyse chain extension with both methylmalonyl- and malonyl-CoA (Funabashi et al., 2008). From these data, it appears that bacterial type III PKS access a richer chemistry than their plant counterparts.

3. Characterisation of type III PKSs in myxobacteria

We aimed to extend the study of bacterial type III PKSs to myxobacteria, by taking advantage of available genome sequence data (Goldman et al., 2006; Schneiker et al., 2007; Ronning and Nierman, 2007). The genome of *S. cellulosum* So ce56 contains two ORFs (*soceCHS1*, sce2133 and *soceCHS2*, sce2182) which putatively encode CHS-like proteins, while a single CHS homologue exists in both *M. xanthus* DK1622 and *S. aurantiaca* DW4/3-1 (MXAN_6639 and STIAU_8629, respectively; the genes share 90% sequence identity, so for the sake of clarity, we will only discuss the CHS from *M. xanthus* DK1622). As indicated by sequence analysis, these type III PKSs belong to three of the five functional categories, suggesting that they serve divergent functions in the bacteria.

SoceCHS1 exhibits significant homology to the THN synthase RppA from *S. griseus* (69% sequence identity), while SoceCHS2 resembles a putative CHS from the methanotrophic bacterium *Methylocella silvestris* (62% identity). The protein encoded by MXAN_6639, termed MxCHS, shows convincing homology to the alkylresorcinol synthases SrsA from *S. griseus* and SCO7671 from *S. coelicolor* (52% and 46% identity, respectively). The dissimilar complement of type III PKS genes in So ce56 and DK1622 is consistent with the substantial evolutionary divergence between these bacteria, as revealed by whole-genome sequencing (Schneiker et al., 2007). Unfortunately, intensive screening of metabolites from both strains did not reveal any compounds that could result from the type III PKS enzymes, indicating the corresponding genes are silent under laboratory conditions. Further support for this