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New Prodiginines from a Ketosynthase Swap

The prodiginine antibiotics exhibit antitumor and immunosuppressive activity. In this issue of *Chemistry & Biology*, Reynolds and coworkers [1] demonstrate that new prodiginines can be obtained by substituting a FabH ketosynthase for the RedP ketosynthase in the undecylprodiginine biosynthetic gene cluster.

The prodiginines are a family of red-pigmented antibiotics produced by Actinomycetes and other eubacteria [2]. These compounds exhibit a broad range of activity against bacteria, protozoa, and pathogenic fungi, but they have not been used clinically because of their toxicity [2, 3]. Interest in the clinical development of prodiginine-like drugs has been stimulated by their novel immunosuppressive activity, which functions by a different mechanism than either cyclosporine or FK506 [2], and by their potent apoptotic effects on cancer cells [4]. A number of new prodiginine analogs with improved therapeutic value have been prepared synthetically [2, 4]. The most thoroughly studied member of the prodiginine family is undecylprodiginine (**1**), which is produced by *Streptomyces coelicolor* A3(2) along with the oxidatively cyclized analog, butyl-m-cycloheptylprodiginine (**2**) (Figure 1). Labeling studies have shown that undecylprodiginine is derived from one unit each of proline, glycine, and serine and multiple units of acetate via a convergent biosynthetic pathway that involves the condensation of of 4-methoxy-2,2′-bipyrrole-5-carboxaldehyde (**3**) with 2-undecylpyrrole (**4**) (Figure 1) [5–7]. Sequencing of the *S. coelicolor* A3(2) genome has revealed the entire *red* biosynthetic gene cluster that is responsible for prodiginine production in this organism [3]. Eight of the 23 genes in the cluster have been proposed to play a role in the formation of 2-undecylpyrrole [3]. Three genes in the 2-undecylpyrrole pathway, *redP*, *redR*, and *redQ*, exhibit homologies to the ketoacyl acyl carrier protein (ACP) synthase FabH (KASIII), the ketoacyl ACP synthase FabF (KASII), and to ACPs, respectively [3]. FabH, FabF, and ACP are components of the type II dissociated fatty acid synthase (FAS) in *Streptomyces*, *Escherichia coli*, and other bacteria [8–11]. The role of FabH in type II FAS systems is to initiate fatty acid biosynthesis by catalyzing the condensation of an acyl-CoA thioester with a malonyl ACP [12], while FabF functions to extend the fatty acid chain by catalyzing subsequent condensation reactions. The acyl group specificity of FabH appears to contribute to the type of fatty acids made by an organism [13–15]. Because of the aforementioned homologies, it has been postulated that RedP initiates 2-undecylpyrrole (**4**) biosynthesis by catalyzing the condensation of acetyl CoA with malonyl RedQ [3]. The resulting acetoacetyl thioester bound to RedQ is then reduced to butyryl RedQ by the appropriate type II FAS enzymes [3]. The FabF homolog RedR is proposed to catalyze four additional elongation steps by using malonyl RedQ, with complete FAS processing of the 3-keto group occurring after each condensation. The resulting dodecyl RedQ is thought to serve as the substrate for an unusual polyketide synthase (PKS) encoded by *redX* that uses a malonyl extender unit bound to an ACP domain in the *redN* gene product to produce a 3-ketomyristoyl thioester. Additional enzymes encoded by the prodiginine gene cluster then convert the 3-ketomyristoyl thioester into 2-undecylpyrrole (**4**) [3].

Since fatty acid biosynthesis and polyketide biosynthesis share similar enzymology, the relationship between these two biosynthetic processes in polyketide-producing organisms such as *Streptomyces* has been the subject of active inquiry [9, 11, 16, 17]. The results of recent studies have revealed that KASs can show ACP specificities that allow these two processes to function independently [16]. It has been found, for example, that the FabH of *Streptomyces* only reacts with FAS ACPs and does not recognize ACPs of type II PKSs [16]. Consequently, the interaction of a FAS-like process with a PKS should involve a specific set of KAS enzymes as well as a dedicated ACP [17]. The presence of FabH homologs in a number of polyketide biosynthetic gene clusters [18, 19] is consistent with this expectation, as is the mechanism proposed for the formation of a dodecyl ACP in prodiginine biosynthesis. The article by Reynolds et al. in this issue of *Chemistry & Biology* [1] demonstrates that the interaction of FAS enzymes with the undecylprodiginine pathway is more complex than anticipated. These investigators have created a *redP*-deletion mutant of *S. coelicolor* and have shown that it produces reduced levels of prodiginines as well as two new analogs, 2-methylundecylprodiginine and 2-methyldodecylprodiginine. Precursor incorporation experiments with perdeuterated valine indicate that these new analogs are produced from 3-methylbutyryl-CoA and isobutyryl-CoA starter units, respectively. The 2-methylundecylprodiginine produced in this mutant results from the extension of the leucine catabolite 3-methylbutyryl CoA with five malonyl units, while the 2-methyldodecylprodiginine is formed by extension of the valine metabolite isobutyryl CoA with six malonyl units. Since the FabH of Streptomycetes has been shown to exhibit a relaxed acyl group substrate specificity compared to the *E. coli* FabH and is able to use branched-chain CoA esters [13], the production of these new alkyl prodiginines can be explained if the priming of prodiginine biosynthesis in the *redP* mutant is initiated by the endogenous *S. coelicolor* FabH. The role of FabH was confirmed by introduction of a plasmid expressing the *S. glaucescens* FabH into the *redP* mutant. The complemented mutant showed a nearly 4-fold increase in overall prodiginine production, while the ratios of the various analogs were unchanged from those of the mutant. Furthermore, introduction of a plasmid expressing either RedP or the *E. coli* FabH into

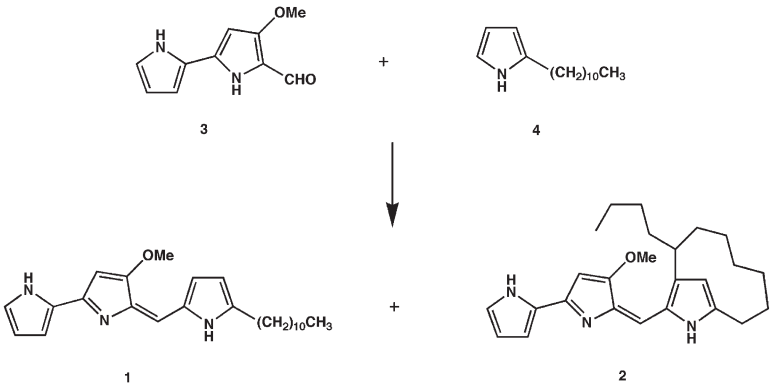

Figure 1. Biosynthesis of Undecylprodiginine and Butyl-m-Cycloheptylprodiginine from 4-Methoxy-2,2′-Bipyrrole-5-Carboxaldehyde and 2-Undecylpyrrole

the mutant reduced the levels of the new, branched prodiginines relative to undecylprodiginine, as would be expected.

An interesting feature of the FabH-dependent prodiginine pathway is that the straight-chain prodiginine analogs predominate. In contrast, the fatty acids of *Streptomyces* consist primarily of branched-chain compounds [20]. This difference could be produced by changes in the pool size of different acyl CoA esters as the fermentation enters stationary phase and prodiginine production begins. On the other hand, it might result from differences in the specificity of downstream enzymes. For example, the fatty acid extension enzyme FabF may prefer branched-chain acyl ACP substrates, while RedR (a FabF homolog) may process straight-chain acyl ACPs more efficiently. Additional studies will clearly be needed to elucidate the reasons for the observed differences in product ratios between fatty acid biosynthesis and FabH-dependent undecylprodiginine biosynthesis. If the ratio of straight-chain to branched-chain prodiginines is governed by the specificity of downstream enzymes, then the engineering of the prodiginine pathway to produce a preponderance of branched-chain analogs could prove to be challenging. Nevertheless, the work of Reynolds et al. [1] is noteworthy since it provides an example of the deployment of a FAS FabH to alter the outcome of a secondary metabolic pathway. This technique should be useful for the production of new analogs of other natural products.

Ronald J. Parry
Department of Chemistry
Rice University
Houston, Texas 77005

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