Kinetic Analysis of the Actinorhodin Aromatic Polyketide Synthase*

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Type II polyketide synthases (PKSs) are bacterial multifunctional enzymes whose biosynthesis of a broad range of natural products. A core set of subunits, consisting of a ketosynthase, a chain length factor, an acyl carrier protein (ACP) and possibly a malonyl CoA:ACP transacylase (MAT) forms a “minimal” PKS. They generate a poly-β-ketone backbone of a specified length from malonyl-CoA derived building blocks. Here we (a) report on the kinetic properties of the actinothorin minimal PKS, and (b) present further data in support of the requirement of the MAT. Kinetic analysis showed that the apoACP is a competitive inhibitor of minimal PKS activity, demonstrating the importance of protein-protein interactions between the polypeptide moiety of the ACP and the remainder of the minimal PKS. In further support of the requirement of MAT for PKS activity, two new findings are presented. First, we observe hyperbolic dependence of PKS activity on MAT concentration, saturating at very low amounts (half-maximal rate at 19.7 ± 5.1 nM). Since MAT can support PKS activity at less than 1/100 the typical concentration of the ACP and ketosynthase/chain length factor components, it is difficult to rule out the presence of trace quantities of MAT in a PKS reaction mixture. Moreover, an S97A mutant was constructed at the nucleophilic active site of the MAT. Not only can this mutant protein support PKS activity, it is also covalently labeled by [14C]malonyl-CoA, demonstrating that the serine nucleophile (which has been the target of PMSF inhibition in earlier studies) is dispensable for MAT activity in a Type II PKS system.

Polypeptides are a large family of structurally diverse natural products which are synthesized by a variety of organisms. They include many macrocyclic and aromatic compounds such as tetracyclines and erythromycin (antibiotics), FK506 (immunosuppressant), doxorubicin (anticancer), and avermectin (antiparasite). The aforementioned are all from actinomycetes, a bacterial group that synthesizes a large proportion of such compounds. Gene clusters of several bacterial and fungal polyketide pathways have been cloned and analyzed. Sequence analysis has revealed that polyketide synthases (PKSs) are multifunctional enzymes that are structurally and mechanistically related to fatty acid synthases. Through a catalytic process involving repeated decarboxylative condensations between coenzyme A-derived acylthioesters, they synthesize a growing carbon chain backbone that is regio- and stereoselectively modified into the final natural product. PKSs achieve enormous structural variety by controlling the overall chain length, choosing primer and extender units (usually acetyl, propionyl, malonyl, and methylmalonyl) and, especially in the case of aromatic polyketides, guiding the regiospecific cyclizations of growing chains (for reviews, see Refs. 1–4).

Type II PKSs are a family of bacterial PKSs related to Type II fatty acid synthases found in bacteria and plants. They catalyze the biosynthesis of a broad range of polyfunctional aromatic natural products. These PKSs contain a single set of iteratively used active sites carried on separate proteins. They consist of a “minimal” PKS and auxiliary subunits. The minimal PKS is composed of four subunits: a ketosynthase (KS), a chain length factor (CLF), an acyl carrier protein (ACP), and possibly a malonyl CoA:ACP transacylase (MAT) (5, 6). To be active the ACP needs a phosphopantetheine arm where acyl chains are attached during the course of polyketide synthesis. Since apoACP, which lacks this prosthetic group, fails to support polyketide synthesis, the role of the pantetheine arm in catalysis has been well established. In contrast, the role (if any) of the polypeptide moiety of the ACP is less clear. Indeed, in certain polyketide synthases and non-ribosomal peptide synthetases, the requirement of an acyl carrier protein can be by-passed by presenting the condensing enzyme with appropriate CoA- or CoA-mimetic substrates (7).

A model has been proposed for the sequence of reactions in aromatic polyketide biosynthesis by the minimal PKS (Fig. 1). Malonyl units are transferred from malonyl CoA to the ACP by the MAT. Repeated decarboxylative condensations occur between the ACP-bound nucleophilic extender units and the KS-bound electrophilic growing chains, giving rise to a poly-β-ketone backbone of a specified length (5). The system is primed by a decarboxylated malonyl unit by a mechanism that remains to be elucidated, but presumably involves the decarboxylative activity of the KS (8). In the case of the actinorhodin (act) minimal PKS, a 16-carbon backbone is generated, which subsequently undergoes cyclization to generate two principal products, SEK4 and SEK4b (Fig. 1).

There is some debate about the requirement of the MAT for minimal PKS activity. Since no MAT or homologous protein is encoded within typical aromatic PKS gene clusters, it was originally suggested that these aromatic PKSs utilized the MAT from primary fatty acid metabolism (9, 10). As a direct test of this idea, we attempted to reconstitute the act minimal PKS from purified components (5). In our laboratory purified KS-CLF (which co-elute as a tight complex) and ACP were protein; MAT, malonyl CoA:ACP transacylase; act, actinorhodin; fren, frenolcin; V, initial velocity; PAGE, polyacrylamide gel electrophoresis.
found to be insufficient for reconstituting PKS activity comparable to that seen in crude cell-free extracts of *Streptomyces coelicolor*, which produces actinorhodin. However, when crude extracts from CH999, an engineered strain of *S. coelicolor* that lacks the entire act gene cluster, were added to purified KS-CLF and ACP, PKS activity was observed. Based on this assay, we purified a 32-kDa protein from CH999 that was responsible for this activity. N-terminal sequencing revealed that it was identical to the MAT involved in fatty acid biosynthesis in *S. coelicolor*. Based on this and earlier in vivo results (9, 10), we proposed that the fatty acid MAT is also a component of the act minimal PKS. A similar conclusion was also reached by Hutchinson and co-workers (6) who determined that >90% of the malonyl-CoA incorporated into TCM F2 was derived from a MAT-catalyzed pathway. However, two recent communications from Simpson and co-workers (11, 12) have challenged the validity of these results. Hitchman, et al. (11) showed that: (i) in contrast to the *Escherichia coli* ACP (obtained from a commercial source and purified) wild-type and mutant forms of the *act* ACP is capable of transferring malonyl groups from malonyl-CoA in the absence of MAT; (ii) that this self-malonylation reaction has a $K_m$ of 0.34 min$^{-1}$ and a $k_m$ of 219 μM; and (iii) that this reaction is not inhibited by phenylmethylsulfonyl fluoride, a known inhibitor of the *E. coli* MAT. These results led them to speculate that an MAT is not required for PKS activity, and that the KS, CLF, and ACP may constitute a truly minimal PKS. Subsequently, Matharu et al. (12) presented additional data in support of this model. They showed that: (i) their ACP and KS/CLF preparations, which contain low (but non-zero) MAT activity, were necessary and sufficient for PKS activity, and (ii) the marginal advantage of the *S. coelicolor* MAT on the rate of polyketide synthesis is only detectable up to a concentration of 20 μM ACP. Thus, the exact composition of the minimal PKS remains unresolved.

This report describes the steady-state kinetic properties of the *act* minimal PKS. The dependence of the rate of polyketide synthesis on the concentration of individual protein components is assessed. Likewise, the ability of the apoACP to inhibit minimal PKS activity is evaluated. Finally, an S97A mutant of the *S. coelicolor* MAT, which lacks the nucleophile on which malonyl groups are believed to be transiently attached, has been constructed and analyzed. Our results provide direct evidence for interactions between the polypeptide portion of the ACP and the remainder of the minimal PKS components. Moreover, they also shed further light on whether physiologically relevant minimal PKS activity requires the MAT or not.

**EXPERIMENTAL PROCEDURES**

**Materials**—[14C]Malonyl-coenzyme A (25 μCi/ml, 52 mCi/mmole) was purchased from Movarek Biochemicals. Silica gel plates Si250F for thin layer chromatography were obtained from J. T. Baker. All other chemicals were purchased from Sigma and were of the highest available grade.

**Protein Expression and Purification**—Both the act KS/CLF and the act holoACP were obtained from *S. coelicolor* CH999/pSKE38 cultures (13). The act KS/CLF complex was purified as described earlier (5, 8). Although Simpson and co-workers (12) have reported difficulties in reproducing these procedures, the published protocols reliably yield KS/CLF preparations that are devoid of ACP and MAT (as judged by SDS-PAGE as well as the absolute requirement of both ACP and MAT for minimal PKS activity). Fractions containing the act holoACP were collected at the beginning of the (NH₄)₂SO₄ gradient from phenyl-Sepharose columns (as described for KS/CLF). Further purification of the ACP was achieved with a Resource Q (Pharmacia) column using a gradient from buffer A (20 mM Tris, pH 6, 2 mM EDTA, 2 mM dithiothreitol, 15% glycerol) to buffer B (A + 1 M NaCl). The ACP eluted between 240 and 300 mM NaCl. Gel filtration on a Superdex-200 column in 100 mM NaHPO₄, pH 7.3, 2 mM EDTA, 2 mM dithiothreitol was performed as the final purification step. Frenolicin (fren) apoACP was purified from an *E. coli* strain expressing this gene as described earlier (14, 15). Fren holoACP was obtained by coexpressing fren ACP and Sfp phosphopantetheinyl transferase (16, 17) in *E. coli*. The sequence for the fabD gene of *S. coelicolor* A3(2) (5), which encodes the MAT, has been deposited in the EMBL Data Base under accession number X86475. The following primers were designed with NdeI and EcoRI sites at the 5’ and 3’ termini, respectively: ATATACATGTGTGTAATGTCCGTAGAC and ATCCGGATTGGCGTGGTAGTCTCCGCG. The stop codon was deleted. *S. coelicolor* genomic DNA was used as a template for polymerase chain reaction. The polymerase chain reaction fragment inserted into pET21c vector in the NdeI-EcoRI restriction sites to result in pANS512. The expressed protein had a 6xHis tag at the C-terminal end for simpler purification. *E. coli* BL21(DE3) cells were transformed with pANS512, containing the cloned *S. coelicolor* MAT gene. 1-Liter cultures were induced with 1 mM isopropyl-thio-β-D-galactopyranoside at $A_{600}$ = 0.6–0.8. Cells were harvested 3 h after induction. The cell pellet was resuspended in 50 mM Tris, pH 8.4, and lysed by sonication on ice. Cell debris was pelleted at 11,000 rpm, 4 °C and the supernatant was loaded onto a nickel NTA-agarose column (Qiagen). MAT elutes at 50 mM imidazole, pH 8.0. Protein was obtained at yields of ~1 mg/liter and was judged to be >95% pure by SDS-PAGE. The MAT was concentrated after purification and buffer was changed to 100 mM NaHPO₄, pH 7.3, 2 mM EDTA, 2 mM dithiothreitol. All protein concentrations were determined by Lowry assays (18) using a kit from Sigma diagnostics.

**PKS Activity Assays**—Production of SEK4 and SEK4b was monitored in vitro by an assay based on [14C]-labeled malonyl-CoA incorporation. 100-μl reactions in 100 mM NaHPO₄, pH 7.3, 2 mM EDTA, 2 mM dithiothreitol were started by adding [14C]-malonyl-CoA (23.654 μM, 0.705 mCi/mmole) to a final concentration of 2.4 mM and stopped by addition of solid NaH₂PO₄. Reaction products were visualized by thin layer chromatography (TLC) as described in Ref. 5. Products were quantified by counting radioactivity using an InstantImager 2024 (Packard).

**Mutagenesis and Analysis of the MAT**—MAT S97A was constructed using pANS512 as template for a T7 DNA polymerase-based double-stranded, site-directed mutagenesis method (Chameleon, Stratagene). The mutant clone used for enzyme preparation was verified by DNA sequencing.

21-μl Reaction mixtures of 25 pmol of MAT and [14C]-labeled malonyl-CoA were incubated in 100 mM NaHPO₄, pH 7.3, 2 mM EDTA, 2 mM dithiothreitol. Products were quantified by counting radioactivity using an InstantImager 2024 (Packard).
concentrations of KS and CLF were calculated assuming an active site for the condensation of acylthioesters. Therefore, a tight complex. One pair of KS and CLF is expected to contain mal PKS is formed by the KS and CLF. These two proteins form addition. In a series of reactions, initial velocities of SEK4 were measured at 20 μM act holoACP, 100 nM MAT, and varying amounts of KS/CLF.

Radiolabeled using [14C]malonyl-CoA as substrate. SEK4 and SEK4b production. Initial velocities of polyketide production were measured at 20 μM act holoACP, 100 nM MAT, and 0.8 μM KS/CLF is linear over about 30 min; during this period the PKS turns over approximately 12 times. We measured SEK4 and SEK4b production within this range to determine initial reaction velocities for different conditions. In a series of reactions, initial velocities of SEK4 + SEK4b production were measured as a function of malonyl-CoA concentration. The apparent $K_m$ for malonyl-CoA—Polyketides produced in vitro can be radiolabeled using [14C]malonyl-CoA as substrate. SEK4 and SEK4b, synthesized by the act minimal PKS, are visualized and quantities determined by TLC autoradiography. Polyketide production in 100-μl reactions containing 20 μM act holoACP, 100 nM MAT, and 0.8 μM KS/CLF is linear over about 30 min; during this period the PKS turns over approximately 12 times. We measured SEK4 and SEK4b production within this range to determine initial reaction velocities for different conditions. In a series of reactions, initial velocities of SEK4 + SEK4b production were measured as a function of malonyl-CoA concentration. The apparent $K_m$ for malonyl-CoA was found to be 79.1 ± 8.2 μM (data not shown). To keep the substrate concentration at saturating levels, all reactions described below were performed at 2.4 mM malonyl-CoA concentrations.

**Titration of KS/CLF**—The core component of the act minimal PKS is formed by the KS and CLF. These two proteins form a tight complex. One pair of KS and CLF is expected to contain one active site for the condensation of acylthioesters. Therefore, concentrations of KS and CLF were calculated assuming an αβ type complex representing 1 catalytic unit even though these two proteins purify as an αβ heterotetramer (5). KS/CLF was titrated into reaction mixtures containing a fixed act holoACP concentration of 20 μM and a fixed MAT concentration of 100 nM. (These concentrations of both MAT and ACP are sufficient to saturate the system, as described below.) Initial reaction velocities were measured up to 1.6 μM KS/CLF, an upper limit dictated by the concentrations of the different protein preparations. When initial reaction velocities were plotted against KS/CLF concentration, a linear relationship was observed (Fig. 2). From the slope of this line, an apparent $k_{cat}$ of 0.31 ± 0.11 min$^{-1}$ was deduced (average based on three independent experiments).

**Titration of MAT**—The MAT was titrated into a reaction mixture containing 0.8 μM act KS/CLF and 20 μM act holoACP (Fig. 3). Several features should be noted. First, within the limits of TLC autoradiography based detection, no PKS activity could be observed in the absence of MAT. Second, MAT is required in only small amounts to allow maximal activity of the system. In an effort to quantify the ability of MAT to saturate the system, we determined the concentration of MAT at which 50% of the maximum velocity was reached. This concentration is found to be 19.7 ± 5.1 nM (average based on two independent experiments). Third, fitting a hyperbolic curve to the data shown in Fig. 3 yielded an apparent $V_{max}$ of 33.2 ± 2.1 pmol/min. Taking KS/CLF as the enzyme in the reaction, an apparent $k_{cat}$ of 0.41 ± 0.03 min$^{-1}$ is calculated, which is in good agreement with the apparent $k_{cat}$ measured in the KS-CLF titrations described above.

**Titration of the Fren ACP**—Fren holoACP was added in increasing amounts to reaction mixtures containing 0.8 μM KS/CLF and 100 nM MAT. The rate of SEK4 and SEK4b production depends on fren holoACP concentration as shown in Fig. 4A. At about 10 μM fren holoACP a maximum velocity is reached. A hyperbolic curve fitted to the data measured shows a $V_{max}$ of 34.7 ± 1.7 pmol/min. Half-maximal velocity is reached at 2.1 ± 0.55 μM.

**Titration of the Act ACP**—The same experiment as with fren ACP was performed with wild-type act holoACP. As shown in Fig. 5, the dependence of the rate of polyketide synthesis on the concentration of act holoACP is complex; it peaks at a concentration of 20 μM and drops thereafter. This profile was reproducibly observed in three separate experiments conducted with independent protein preparations. The reaction velocity at 20 μM ACP is 34 pmol/min, corresponding to an apparent $k_{cat}$ of 0.43 min$^{-1}$, which compares well with the numbers obtained in MAT and KS/CLF titrations. Not accounting for the inhibitory effects that are observed beyond 20 μM (see “Discussion”), a half-saturating concentration for ACP can be estimated to be approximately 5 μM (average based on three independent experiments).

**Inhibition of Polyketide Synthesis by ApoACP**—Fren holoACP titrations were performed as described above (0.8 μM KS/CLF, 100 nM MAT). In these experiments the fren apoACP was present in the reaction at a fixed concentration. Interestingly, we observe a clear inhibition of SEK4 and SEK4b production in the presence of the catalytically inactive apoACP. The extent of inhibition depends on apoACP concentration. Thus, we determined the effect of apoACP by calculation of $K_i$ values for competitive and noncompetitive inhibition. For the competitive component a $K_i$ of 12.3 μM is deduced (Fig. 4B). As the noncompetitive part leads to a very high $K_i$ (> 300 μM), no significant noncompetitive competition is measured. To confirm that fren apoACP also inhibits activity of the PKS in the presence of the act holoACP, the experiment was repeated by varying the concentration of the act holoACP in the presence of 6 μM fren apoACP. Again, significant inhibition by apoACP was observed in this case (Fig. 5); however, given the unusual features of the titration curve for the act holoACP in the absence of apoACP, quantitative measurements of inhibitory constants were not made.

**Construction and Analysis of the S97A Mutant of the MAT**—
Extensive analysis of acyltransferases from various fatty acid or polyketide synthases has shown that a serine residue corre-
Kinetic Analysis of an Aromatic Polyketide Synthase

Fig. 4. A, initial velocities of SEK4 and SEK4b production were measured for 0.8 \( \mu \text{M} \) KS/CLF, 100 nM MAT and fren holoACP concentrations from 0 to 35 \( \mu \text{M} \). A hyperbolic curve was fitted to the data measured. B, apoACP inhibition of fren holoACP. \( K_{\text{apoACP}} \) values are holoACP concentrations required to reach \( V_{\text{max}}/2 \) in the presence of different amounts of apoACP. The linear fit was used to calculate a \( K_{i} \) for competitive inhibition.

Fig. 5. Act holoACP titration. Initial velocities of SEK4 and SEK4b production for 0.8 \( \mu \text{M} \) KS/CLF, 100 nM MAT, and act holoACP concentrations from 0 to 35 \( \mu \text{M} \) were determined (closed circles). The same experiment was performed in the presence of 6 \( \mu \text{M} \) apoACP (open circles).

Fig. 6. Analysis of MAT S97A. Panel A shows the results of SDS-PAGE autoradiography in the presence of \([^{14}\text{C}]\text{malonyl-CoA} \), as described under “Experimental Procedures.” Lane 1, wild-type MAT; lane 2, MAT S97A. Panel B shows an autoradiograph of a TLC plate used to develop extracts from \textit{in vitro} PKS assay mixtures. All lanes show products from 100-\( \mu \text{L} \) assays run for 2 h. All samples shown contain 0.8 \( \mu \text{M} \) act KS/CLF, 20 \( \mu \text{M} \) fren holoACP, and the following additions of MAT: lane 1, wild-type MAT; lane 2, 1 \( \mu \text{M} \) MAT S97A; lane 3, no MAT. In lane 3 one observes trace quantities of polyketide production; however, the kinetics of this MAT-independent process are difficult to accurately measure, and are estimated to be less than 5% of the MAT-dependent process (see text).

DISCUSSION

Since the genetic characterization of bacterial aromatic polyketide synthases (13, 20–27), their mechanisms and relationships with Type II fatty acid synthases have been of considerable interest. Initial efforts toward these goals relied on metabolic characterization of genetically manipulated PKSs. More recently, cell-free systems have been developed to study polyketide synthesis \textit{in vitro} (5, 6, 8). The actinorhodin (act) PKS from \textit{S. coelicolor} has been an excellent model system for both \textit{in vivo} and \textit{in vitro} studies.

Here we have quantified the steady-state kinetic properties of this minimal PKS. In particular, titrations of the individual components of the minimal PKS have yielded interesting insights into the properties of Type II PKSs. At saturating concentrations of ACP and MAT, the dependence of initial reaction velocity on KS/CLF concentration is apparently linear up to a KS/CLF concentration of 1.6 \( \mu \text{M} \) (Fig. 2). The reaction rate could be expected to saturate at higher KS/CLF concentrations; however, practical limitations prevented the direct observation of this saturation. An apparent \( k_{\text{cat}} \) of 0.31 min\(^{-1}\) was derived from these measurements, which is in good agreement with an apparent \( k_{\text{cat}} \) of 0.41 min\(^{-1}\) derived from MAT titrations (Fig. 3), as well as with recently reported measurements in the range of 1 min\(^{-1}\) (12).

Similar titration analysis was also performed for the act ACP (Fig. 5). At 100 nM MAT and 0.8 \( \mu \text{M} \) KS/CLF, the reaction rate increased with increasing amounts of ACP up to a concentration of 20 \( \mu \text{M} \), and decreased thereafter. The reason for this inhibition is unclear, and may perhaps reflect the ability of the wild-type act ACP to dimerize at high concentrations (12). However, because of the inhibition observed at high ACP concentrations, all other measurements in this study involving the act holoACP were made at a holoACP concentration of 20 \( \mu \text{M} \).
The fren ACP saturates the multicomponent system at slightly lower concentrations than act ACP (Fig. 4). Inhibition is not observed at higher fren ACP concentrations.

The observation that ACP is needed in substantially more than stoichiometric amounts compared with the KS/CLF for maximal activity is consistent with earlier genetic studies where alterations in expression levels of the ACP gene resulted in significant differences in polyketide production in vivo (28). It suggests that the ACP interacts only loosely with the PKS core comprised of the KS and CLF. However, ACP titrations alone do not allow discrimination between two alternative models for Type II PKS activity, a “static” model in which one ACP molecule interacts with a given KS/CLF pair to chaperone the synthesis of a complete octaketide chain, versus a “dynamic” model in which KS/CLF and ACP proteins can interchange during the overall catalytic cycle. More detailed kinetic studies should be illuminating in this regard.

Three results described here shed new light on the debate over whether the MAT is required for minimal PKS activity or not. First, although the $k_{cat}$ reported here for the (MAT-containing) act PKS is similar to the $k_{cat}$ measured for the self-malonation reaction of the act ACP (0.34 min$^{-1}$) (11), the $K_m$ values for malonyl-CoA in the two reactions are different. Whereas the overall $K_m$ for the (MAT-containing) act PKS is 79 μM, the $K_m$ for the self-malonation reaction is 216 μM. Since intracellular malonyl-CoA concentrations are relatively low, this difference supports the notion that the MAT-dependent pathway dominates over the MAT-independent one in vivo. Second, a crucial titration reported here is that of the MAT component into the PKS reaction mixture (Fig. 3). In this context it is important to note that we do not detect any PKS activity in the absence of the MAT at a KS/CLF concentration of 0.8 μM and an act or fren holoACP concentration of 20 μM (Fig. 3). (Under these conditions, our assays can reliably detect PKS activity that is as little as about 5% of the maximum reaction velocity.) This is consistent with our earlier observations (5), as well as those of Hutchinson and co-workers (6) made with the tetracenomycin PKS, but contradicts the observations of Simpson and co-workers (12). More importantly, unlike KS/CLF and ACP, MAT is required in very small amounts for maximal PKS activity. $V_{max}/2$ is reached at a MAT concentration of 19.7 nM and suggests a very fast or tight interaction with the other PKS components. The role of the MAT is to acylate the ACP with malonyl units derived from malonyl-CoA. Low MAT concentrations required to saturate the minimal PKS most probably result from highly efficient acylation of the ACP by the MAT, followed by a slower condensation reaction between the KS- and ACP-bound substrates. Work on fatty acid synthesis where turnover rates as high as 1.6 × 10$^8$ s$^{-1}$, were measured for the MAT, support this view (19). Finally, our studies on the S97A mutant of the $S. coelicolor$ MAT demonstrate that, even when the catalytic potency of the MAT is drastically attenuated by removal of the active site nucleophile, MAT can support PKS activity, perhaps through the recruitment of an alternative covalent attachment site for malonyl extender units. Based on these results, we propose that MAT is an important component of the minimal PKS in vivo. A definitive test of this matter would involve the construction and analysis of a knock-out of the MAT gene in $S. coelicolor$; however, since MAT is also required for fatty acid biosynthesis in the cell, this is a challenging task.

Perhaps most interestingly, titration of holoACP in the presence of an apoACP revealed that apoACP has a substantial inhibitory effect on SEK4 and SEK4b production (Figs. 4 and 5). In vivo studies in E. coli have recently shown that overexpression of apoACP inhibits growth, although no direct molecular explanation can be derived from these studies (29). Moreover, it is known that E. coli maintains its apoACP concentration at a very low level, perhaps because of its inhibitory effect (30). Since apoACP lacks a pantetheine arm and is catalytically inactive, our results provide direct evidence for functionally relevant interactions between the peptidic portion of a Type II ACP and the remainder of the PKS. A mainly competitive type of inhibition was deduced because apoACP clearly affects saturation and $V_{max}$ only to a lesser extent. Importantly, inhibition is observed with both act and fren holoACP, ruling out a fren-specific phenomenon. It is unclear whether this inhibition reflects interactions between the apoACP protein and MAT or KS/CLF or both. Since the solution structures of several Type II ACPs have been solved (31–38), further studies into the structural basis for these molecular recognition properties could provide fundamentally new insights into the importance of protein-protein interactions in regulating Type II PKS function and specificity.

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