Utilization of Enzymatically Phosphopantetheinylated Acyl Carrier Proteins and Acetyl−Acyl Carrier Proteins by the Actinorhodin Polyketide Synthase†

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ABSTRACT: The functional reconstitution of two purified proteins of an aromatic polyketide synthase pathway, the acyl carrier protein (ACP) and holo-ACP synthase (ACPS), is described. Holo-ACPs were enzymatically synthesized from coenzyme A and apo-ACPs using *Escherichia coli* ACPS. Frenolicin and granaticin holo-ACPs formed in this manner were shown to be fully functional together with the other components of the minimal actinorhodin polyketide synthase (*act* PKS), resulting in synthesis of the same aromatic polyketides as those formed by the *act* PKS *in vivo*. ACPS also catalyzed the transfer of acetyl-, propionyl-, butyryl-, benzoyl-, phenylacetyl-, and malonylphosphopantetheines to apo-ACPs from their corresponding coenzyme As, as detected by electrophoresis and/or mass spectrometry. A steady state kinetic study showed that acetyl-coenzyme A is as efficient an ACPS substrate as coenzyme A, with k_{cat} and K_m values of 20 min^{-1} and 25 μM, respectively. In contrast to acetyl-coenzyme A, enzymatically synthesized acetyl-ACPs were shown to be efficient substrates for the *act* PKS, indicating that acetyl-ACP is a chemically competent intermediate of aromatic polyketide biosynthesis. Together, these methods provide a valuable tool for dissecting the mechanisms and molecular recognition features of polyketide biosynthesis.

Aromatic polyketide synthases (type II PKSs)[1] are a family of enzymes that catalyze the biosynthesis of a structurally diverse and medicinally significant class of natural products known as bacterial aromatic polyketides (Carreras et al., 1997; Hopwood et al., 1993; Hutchinson & Fujii, 1995; Katz & Donadio, 1993). These enzyme systems use a minimum of three proteins, an acyl carrier protein (ACP), ketosynthase (KS), and chain-length factor (CLF), to direct the synthesis of polyketide chains from malonyl-CoA-derived acetate units. The ACP undergoes covalent post-translational modification with a CoASH-derived 4′-phosphopantetheine prosthetic group, which is added to the protein through the action of a holo-ACP synthase (ACPS) (Lambalot et al., 1996). The growing polyketide chain is escorted through synthesis covalently bound to the ACP as a 4′-phosphopantetheine thioester. The KS and CLF are homologous, cotranscribed proteins which are thought to act together to catalyze the decarboxylative condensation of malonyl extender units with the growing polyketide chain. While both the KS and CLF are homologous with respect to the KS proteins involved in bacterial fatty acid synthesis, active site residues that are known to play catalytic roles in fatty acid synthesis are conserved only in the KS (Siggaard-Andersen, 1993). The CLF lacks these conserved residues and has been suggested to play a role in determining the number of condensations catalyzed by the KS protein, and thus in determining polyketide chain length (McDaniel et al., 1993a,b, 1994). In addition to the KS, CLF, and ACP, "downstream" enzymes, including ketoreductases, specific cyclases, and methylases, are used in PKS systems to tailor the polyketide backbone into the final, bioactive product. Genetic manipulation of bacterial aromatic PKSs has provided valuable information about the functions and molecular recognition features of the individual PKS proteins and has resulted in the generation of numerous engineered *Streptomyces* strains which produce novel polyketides [cf. McDaniel et al. (1993a, 1995)]. However, many basic aspects of the structure and mechanism of PKSs remain unknown due to difficulties in reconstitution of the system from purified components.

Here, we demonstrate that several different *Streptomyces* ACPs may be phosphopantetheinylated *in vitro* by purified *Escherichia coli* ACPS and function in polyketide synthesis in conjunction with *act* KS/CLF (Figure 1A). We also show that ACPS catalyzes the transfer of acetyl-, propionyl-, butyryl-, benzoyl-, phenylacetyl-, and malonylphosphopantetheines to apo-ACPs from their corresponding CoA thioesters and that the specificity of ACPS for acetyl-CoA is similar to that for CoASH. Lastly, we demonstrate that, in contrast to acetyl-CoA, acetyl groups of acetyl-ACPs are quantitatively incorporated into known polyketide products through the action of the *act* KS/CLF (Figure 1B). Together, these

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[1] Abbreviations: PKS, polyketide synthase; ACP, acyl carrier protein; ACPS, holo-ACP synthase; CoASH, coenzyme A; KS, ketosynthase; CLF, chain-length factor; *act*, actinorhodin; *gra*, granaticin; *fren*, frenolicin; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; TCA, trichloroacetic acid; BSA, bovine serum albumin; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography; MALDI-TOF, matrix-assisted laser desorption ionization time of flight.

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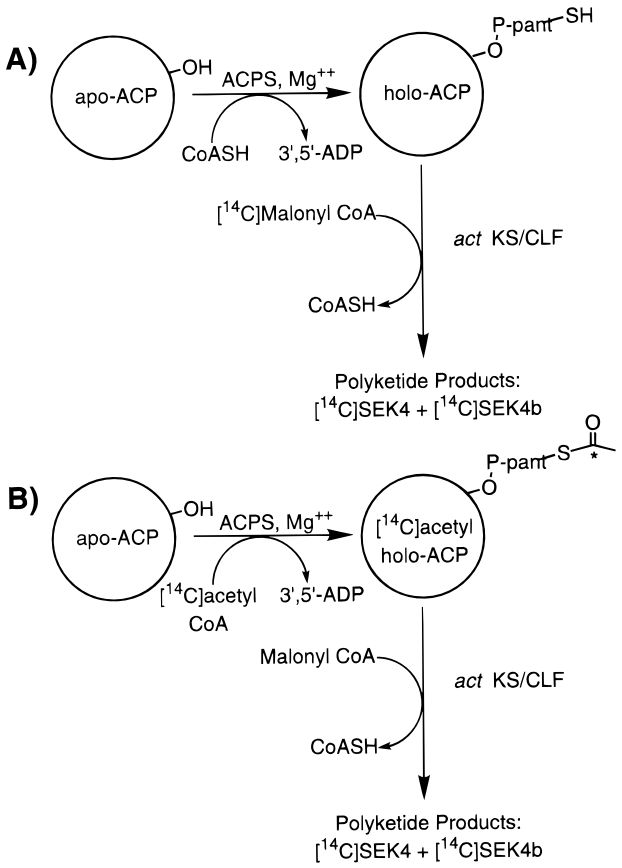

FIGURE 1: Use of ACPS in the formation of functional holo-ACPs. (A) *In vitro* synthesis of [^{14}C]SEK4 and [^{14}C]SEK4b from [^{14}C]-malonyl-CoA catalyzed by *act* KS/CLF and holo-ACP formed by reaction of apo-ACP with CoASH in the presence of ACPS. (B) *In vitro* synthesis of [^{14}C]SEK4 and [^{14}C]SEK4b from malonyl-CoA catalyzed by *act* KS/CLF and [^{14}C]acetyl-ACP formed by reaction of apo-ACP with [^{14}C]acetyl-CoA in the presence of ACPS.

methods provide an important tool for the study of the interaction of ACPs with the other PKS proteins and suggest that the active sites involved in polyketide chain elongation might have a relatively broad specificity toward artificially introduced un-natural primer units.

MATERIALS AND METHODS

Materials. Cell-free extracts of *Streptomyces coelicolor* CH999/pSEK38 and pRM26 [an unpublished derivative of pRM5 (McDaniel et al., 1993a) containing an S42A mutation in the gene encoding the ACP] were prepared as previously described (Carreras et al., 1996). *Act* KS/CLF, stripped of ACP and partially purified, was obtained from protein extracts of *S. coelicolor* CH999/pSEK38 (C. Carreras and C. Khosla, manuscript in preparation). Apo-ACPs were prepared from extracts of *E. coli* BL21DE(3) containing either pET22b-fren ACP or pET22b-gra ACP by freeze/thaw lysis (Morris et al., 1993) and Resource Q chromatography (6 mL, Pharmacia) as previously described (Gehring et al., 1997). The *E. coli* ACPS was purified from *E. coli* BL21DE(3)/pET22b-dpj as previously described (Lambalot & Walsh, 1995). [^{14}C]Malonyl-CoA (56 Ci/mol), [^{14}C]-propionyl-CoA (54 Ci/mol), and [^{14}C]acetyl-CoA (54 Ci/mol) were obtained from Moravek Biochemicals (Brea, CA). [^{14}C]Butyryl-CoA (4 Ci/mol) was obtained from Dupont-NEN. CoASH, malonyl-CoA, acetyl-CoA, benzoyl-CoA, phenylacetyl-CoA, and commonly available chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) and used without purification.

ACPS-Catalyzed Formation of Functional Holo-ACPs. Purified *fren* and *gra* apo-ACPs were quantitated by UV spectroscopy (Gehring et al., 1997) and phosphopantetheinylated in reaction mixtures (20−200 μL) containing 35 μM *fren* or *gra* apo-ACP, 250 nM ACPS, 350 μM CoASH, 50 mM Tris (pH 8.8), 2 mM DTT, 2 mM EDTA, and 10 mM $MgCl_2$ for 2 h at 37 °C. Aliquots (50 μL) from these reaction mixtures were used to complement polyketide synthesis in reaction mixtures (150 μL) containing ~1 μM *act* KS/CLF, 1 mM [^{14}C]malonyl-CoA (0.5 Ci/mol), 100 mM phosphate (pH 7.3), 2 mM DTT, and 2 mM EDTA. Incubations were performed at room temperature for 2 h, and polyketide products were extracted and subjected to TLC and HPLC as previously described (Carreras et al., 1996). Electronic autoradiography was performed using an InstantImager (Packard Instruments).

Formation and Analysis of Acyl-ACPs. Purified *fren* or *gra* apo-ACP was [^{14}C]acylphosphopantetheinylated in reaction mixtures (10 μL) containing 50 μM apo-ACP, 500 nM ACPS, 50 mM phosphate (pH 7.3), 2 mM DTT, 2 mM EDTA, and 10 mM $MgCl_2$ for 2 h at 37 °C. [^{14}C]Acyl-CoAs (4−56 Ci/mol) were included between 300 and 800 μM, depending upon the concentration of the commercially available stock solutions. Qualitative confirmation of the covalent attachment of [^{14}C]acyl groups to the ACP was obtained by subjecting the reaction mixtures to 20% native PAGE or SDS−PAGE, followed by autofluorography. MALDI-TOF mass spectrometry (VG/Fisons TOF mass spectrometer) was used to determine masses of the *gra* acetyl-ACP and the *fren* acetyl-, phenylacetyl-, and benzoyl-ACPs (Howard Hughes Medical Institute Biopolymer Facility, Harvard Medical School). For quantitative determination of the yield in large scale [^{14}C]acetylphosphopantetheinylation reactions, [^{14}C]acetyl-ACP was subjected to HPLC (Gehring et al., 1997) and the amount of ^{14}C coeluting with the holo-ACP was measured by liquid scintillation counting (Beckman LS3801 scintillation counter, Packard Formula 989 Scintillant).

Kinetic Paramaters of ACPS-Catalyzed Acetylphosphopantetheinylation of Fren Apo-ACP. The radioassay for acetylphosphopantetheine transfer was similar to the previously described assay for phosphopantetheine transfer (Gehring et al., 1997). Triplicate reactions (50 μL) with 50 μM *fren* apo-ACP, 75 mM Tris (pH 7.5), 10 mM $MgCl_2$, 5 mM DTT, and 5−200 μM [^{14}C]acetyl-CoA (56 Ci/mol) were initiated by addition of 25 nM ACPS, allowed to proceed for 5 min at 37 °C, and quenched with 800 μL of 10% TCA. BSA (375 μg) was added as a carrier, and the precipitated protein was pelleted by centrifugation. The pellet was washed three times with 10% TCA and dissolved in 1 M Tris base, and the amount of ^{14}C incorporated into the solubilized protein was determined by liquid scintillation counting.

Incorporation of [^{14}C]Acetyl Groups from [^{14}C]Acetyl-ACP into Polyketide Products. Purified *fren* or *gra* apo-ACP was [^{14}C]acetylphosphopantetheinylated in reaction mixtures (500 μL) containing 250 μM apo-ACP, 300 nM ACPS, 375 μM [^{14}C]acetyl-CoA (56 Ci/mol), 50 mM phosphate (pH 7.3), 2 mM DTT, 2 mM EDTA, and 10 mM $MgCl_2$ for 2 h at 37 °C. Unreacted [^{14}C]acetyl-CoA was removed from [^{14}C]acetyl-ACP and unmodified apo-ACP by

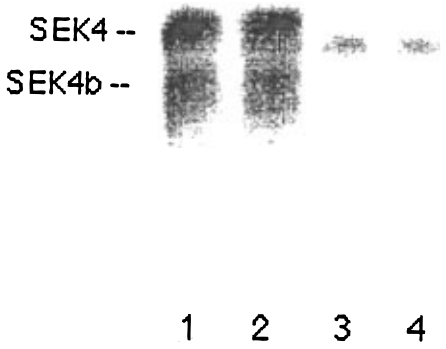

FIGURE 2: Complementation of PKS activity by enzymatically synthesized holo-ACPs. Thin layer chromatogram of the ^{14}C-labeled products formed in reaction mixtures containing [^{14}C]malonyl-CoA, ACP-deficient *act* KS/CLF, and either *fren* apo-ACP (lanes 1 and 3) or *gra* apo-ACP (lanes 2 and 4) that were preincubated with CoASH in the presence (lanes 1 and 2) or absence (lanes 3 and 4) of *E. coli* ACPS. Note that the small amount of radioactive product in lanes 3 and 4 is also formed in extracts of the host strain without plasmid and does not comigrate with SEK4 or SEK4b on HPLC.

Sephadex G-25 chromatography (PD-10, Pharmacia). Half of each 0.5 mL fraction collected was tested for its ability to complement polyketide synthesis in reaction mixtures (500 μL) containing ~1 μM *act* KS/CLF, 1 mM malonyl-CoA, 1.5 mM acetyl-CoA, 100 mM phosphate (pH 7.3), 2 mM DTT, and 2 mM EDTA. To verify that the amount of [^{14}C]-acetyl-CoA contaminating the [^{14}C]acetyl-ACP-containing fractions was too low to account for the [^{14}C]polyketides formed under these conditions, fractions eluting from the PD-10 column after the ACPs (which have a larger amount of [^{14}C]acetyl-CoA) were assayed in PKS reactions in the presence of 10 μM *fren* holo-ACP. Incubations were performed at room temperature for 2 h, and the formation of polyketide products, if any, was examined by TLC and HPLC as previously described (Carreras et al., 1996).

The efficiency of incorporation of [^{14}C]acetyl starter units derived from [^{14}C]acetyl-ACP into [^{14}C]SEK4 and [^{14}C]-SEK4b was determined by varying the amount of [^{14}C]acetyl-ACP between 0 and 0.5 μM in 1.2 mL reaction mixtures containing ~1 μM *act* KS/CLF, 1 mM malonyl-CoA, 1.5 mM unlabeled acetyl-CoA, 100 mM phosphate (pH 7.3), 2 mM DTT, and 2 mM EDTA and measuring the yield of [^{14}C]-SEK4 and [^{14}C]SEK4b that was extracted, separated by TLC, and quantitated by electronic autoradiography.

RESULTS

ACPS-Catalyzed Formation of Functional Holo-ACPs. Homogeneous (>95%) preparations of the *fren* and *gra* apo-ACPs and *E. coli* ACPS were purified from *E. coli* fermentations (Gehring et al., 1997; Lambalot & Walsh, 1995). Treatment of apo-ACPs with CoASH and ACPS resulted in the formation of holo-ACPs that were detected by their ability to complement ACP-deficient *act* KS/CLF preparations in PKS-catalyzed reaction mixtures containing [^{14}C]malonyl-CoA (Figure 2). Phosphopantetheinylation of the apo-ACPs required both CoASH and ACPS, and when they were omitted, no complementation of PKS activity was observed. When either *fren* or *gra* holo-ACPs formed in this manner were combined with the *act* KS/CLF, the major polyketide products of this reaction were identified as SEK4 and SEK4b on the basis of their comigration with authentic standards on both normal phase TLC and ion pair reversed phase HPLC (data not shown); the validity of these analytical procedures was established earlier (Carreras et al., 1996).

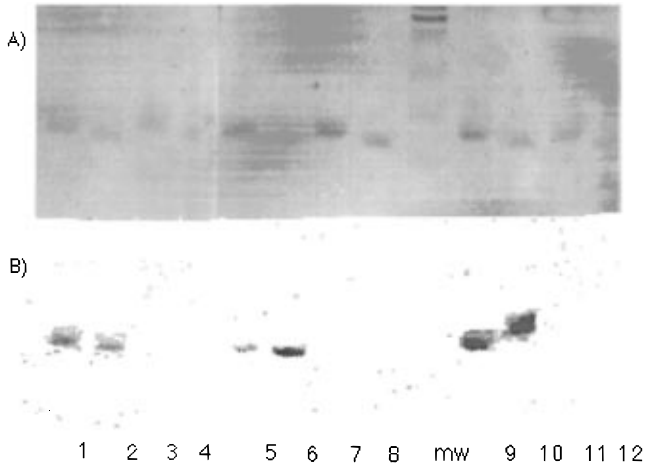

FIGURE 3: ACPS-catalyzed [^{14}C]acylphosphopantetheinylation of apo-ACPs. (A) SDS−PAGE (20%) of labeling reactions. Reactions in even lanes contain *fren* ACP, and those in odd lanes contain *gra* ACP. Reactions in lanes 1 and 2 contain [^{14}C]propionyl-CoA and ACPS, lanes 3 and 4 [^{14}C]propionyl-CoA only, lanes 5 and 6 [^{14}C]butyryl-CoA and ACPS, lanes 7 and 8 [^{14}C]butyryl-CoA only, lanes 9 and 10 [^{14}C]malonyl-CoA and ACPS, lanes 11 and 12 [^{14}C]-malonyl-CoA only. (B) Autoradiograph of the same gel.

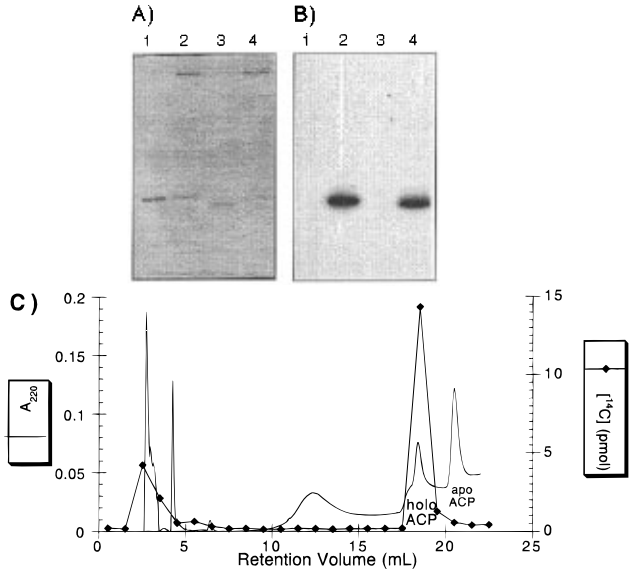

FIGURE 4: ACPS-dependent covalent labeling of apo-ACPs by [^{14}C]-acetyl-CoA. (A) Native PAGE (20%) of *fren* apo-ACP (lanes 1 and 2) and *gra* apo-ACP (lanes 3 and 4) that were incubated with [^{14}C]acetyl-CoA in the presence (lanes 2 and 4) or absence (lanes 1 and 3) of *E. coli* ACPS. (B) Autoradiograph of the same gel. (C) HPLC trace showing comigration of the *fren* [^{14}C]acetyl-ACP coeluting with the *fren* holo-ACP UV peak.

Formation and Analysis of Acyl-ACPs. When purified *fren* or *gra* apo-ACPs were incubated with [^{14}C]acetyl-CoA, [^{14}C]-propionyl-CoA, [^{14}C]butyryl-CoA, or [^{14}C]malonyl-CoA in the presence of purified ACPS, the radioactive labels were transferred to the ACP and detected on 20% SDS−PAGE (Figure 3) or 20% native PAGE (Figure 4A,B). Labeling of the ACPs was only observed when ACPS was included, indicating that ACPS catalyzes the covalent linkage between the labeled acyl group and the apo-ACP. Labeled ACPs comigrated with the corresponding unlabeled apo-ACPs, with the exception of the *gra* [^{14}C]malonyl-ACP, which migrated slightly more slowly than nonacylated forms of the *gra* ACP.

Table 1: Kinetic Parameters for Acetyl-CoA as an ACPS Substrate

substrate	k_{cat} (min^{-1})	K_m (μM)
acetyl-CoA	20	25
CoASH[a]		50
fren apo-ACP[b]	19	12

[a] Lambalot and Walsh (1995). [b] Gehring et al. (1997).

Table 2: Mass Spectral Data for Acyl-ACPs

ACP species	expected mass (Da)	observed mass (Da)
fren apo-ACP	8664	8678
fren acetyl-ACP	9045	9059 (9018)[a]
fren benzoyl-ACP	9107	9108 (9004)[a]
fren phenylacetyl-ACP	9121	9158 (9039)[a]
gra apo-ACP	8860	8873
gra acetyl-ACP	9241	9238 (9194)[b]

[a] Mass of the *fren* holo-ACP peak which appeared along with the acyl-ACP peak in each spectrum. The expected mass for the *fren* holo-ACP is 9003 Da. [b] Mass of the *gra* holo-ACP peak in the same spectrum. The expected mass for the *gra* holo-ACP is 9199 Da.

HPLC analysis of [^{14}C]acetylphosphopantetheinylation reactions using conditions that resolve apo-ACP from holo-ACP showed ^{14}C coeluting with the UV-active holo-ACP peak (Figure 4C). Quantitation of the label by scintillation counting revealed that 2% of the ACP included in the reaction was isolated as [^{14}C]acetyl-ACP. No attempt was made to optimize the yield of this reaction.

Because ^{14}C-labeled benzoyl- and phenylacetyl-CoAs are not readily available, the products of their reaction with apo-ACPs and ACPS were detected by MALDI-TOF mass spectrometry. As shown in Table 2, incubation of *fren* or *gra* apo-ACPs with ACPS and acetyl-, benzoyl-, or phenylacetyl-CoA led to an increase in the mass of the ACP corresponding to the covalent addition of acylpantetheine moieties. In each case, a peak corresponding to desacyl holo-ACP was also observed, presumably arising from hydrolysis of the acyl-CoA (or acyl-ACP) thioester during the incubation period (data not shown). The differences in mass between acyl-ACP and holo-ACP peaks (Table 2 footnote) correspond to the mass of each acyl group; for example, the molecular mass difference between the *fren* phenylacetyl-ACP and desphenylacetyl holo-ACP was observed to be 119 where 118 is the expected difference.

Kinetic Parameters of ACPS-Catalyzed Acetylphosphopantetheinylation of Fren Apo-ACP. The k_{cat} and K_m values for the ACPS-catalyzed transfer of acetylphosphopantetheine from acetyl-CoA to the *fren* apo-ACP are similar to those for the transfer of CoASH-derived phosphopantetheine (Table 1). Thus, acetyl-CoA appears to be as efficient a substrate for ACPS as CoASH.

Incorporation of [^{14}C]Acetyl Groups into Polyketide Products. When *fren* and *gra* [^{14}C]acetyl-ACPs were incubated with malonyl-CoA and *act* KS/CLF, the ^{14}C-labeled products of the reaction comigrated with authentic samples of SEK4 and SEK4b on both ion pair reversed phase HPLC and normal phase TLC. The amount of [^{14}C]SEK4 plus [^{14}C]SEK4b produced in these reactions increased linearly as the amount of [^{14}C]acetyl-ACP increased, and quantitative incorporation of [^{14}C]acetyl-ACP-derived [^{14}C]-acetate into [^{14}C]polyketides was observed (Figure 5).

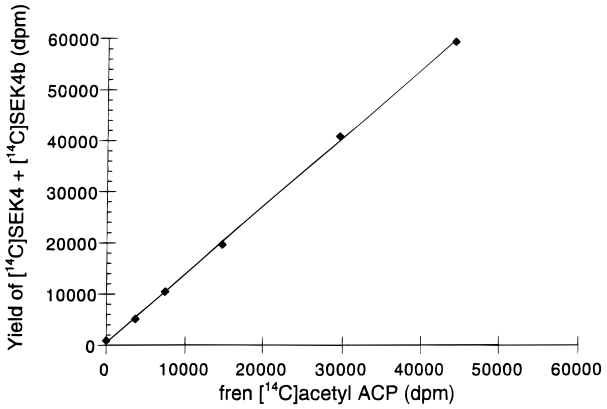

FIGURE 5: Quantitative conversion of [^{14}C]acetyl-ACP into [^{14}C]-SEK4 and [^{14}C]SEK4b. In this plot, the maximum yield of 60 000 dpm corresponds to approximately 0.5 nmol of polyketide product.

DISCUSSION

Building on our previous reports of efficient *in vitro* polyketide (Carreras et al., 1996) and holo-ACP synthesis (Gehring et al., 1997), we describe here the functional reconstitution of two purified proteins of the aromatic polyketide pathway, the ACP and ACPS, and demonstrate their use in a novel *in vitro* pathway in which starter units for polyketide synthesis are loaded directly onto apo-ACPs as acylphosphopantetheines. Thus, the specificity of acyltransferases which load acyl groups onto holo-ACPs in the normal polyketide pathway is bypassed (Figure 1).

Recently, we have succeeded in partially purifying *act* KS/CLF that is devoid of ACP (C. Carreras and C. Khosla, manuscript in preparation). The PKS activity of this preparation can be restored through the addition of exogenous holo-ACP. Here, we have used this partially purified protein preparation to assess the biosynthetic activity of holo- and acetyl-ACPs. *Fren* and *gra* apo-ACPs, overexpressed in *E. coli* and purified to homogeneity, were phosphopantetheinylated *in vitro* through the action of purified *E. coli* ACPS. These holo-ACPs were shown to be fully functional in polyketide synthesis when combined with ACP-deficient *act* KS/CLF proteins isolated from *S. coelicolor*. When either *fren* or *gra* holo-ACPs were used, the products of the reaction were SEK4 and SEK4b, the same products observed *in vivo* for the *act* PKS. As previously indicated from genetic studies (Khosla et al., 1993), with respect to the polyketide yield, the *act* KS/CLF functions equally well with holo-ACPs derived from either the *act*, *fren*, or *gra* PKS systems. In accord with genetic studies of the phosphopantetheinylation-blocked S42A mutant of the *act* ACP (Khosla et al., 1992), apo-ACPs were unable to complement polyketide synthesis. Thus, although holo-ACPs are an essential component of PKS activity, the major determinant of the structure of aromatic polyketide products both *in vivo* and *in vitro* seems to be the KS/CLF.

A recent report has shown that, in the presence of added *act* apo-ACP, [^{14}C]acetyl-CoA-derived radioactivity may be precipitated from crude extracts containing ACPS, indicating that ACPS catalyzes the transfer of acetylphosphopantetheine onto the apo-ACP (Cox et al., 1997). Working with purified apo-ACP and ACPS, we have directly observed the ACPS-catalyzed acetylphosphopantetheinylation of apo-ACPs and have unambiguously identified acetyl-ACP as the product

of this reaction using several methods. First, we observed *fren* and *gra* [^{14}C]acetyl-ACPs as distinct labeled bands on SDS and native PAGE. Second, we isolated [^{14}C]acetyl-ACPs by HPLC, where they are efficiently separated from apo-ACPs and coelute with holo-ACPs. Third, we have identified the product of the acetylphosphopantetheinylation reaction as acetyl ACP by mass spectrometry.

Next, we measured the steady state kinetic paramaters for the ACPS-catalyzed reaction of apo-ACPs with acetyl-CoA. As shown in Table 1, the specificity and efficiency of ACPS are similar toward either CoASH or acetyl-CoA, indicating that the acyl group is tolerated by ACPS without penalty. Given the ready availability of radiolabeled acetyl-CoA while radiolabeled CoASH is currently available only through custom synthesis, acetyl-CoA should be a convenient reagent for measuring phosphopantetheinyltransferase activity. Finally, we demonstrated that *E. coli* ACPS catalyzes the attachment of propionyl-, butyryl-, benzoyl-, phenylacetyl-, and malonylphosphopantetheines onto apo-ACPs from the corresponding CoAs. Thus, a variety of acyl groups, at least as large as phenylacetate and one containing a negatively charged carboxyl group, may be attached directly to apo-ACPs using ACPS.

Enzymatically generated holo- and acyl-ACPs are a valuable tool for mechanistic studies of aromatic polyketide biosynthesis. As an illustration of their utility, acetyl-ACPs were shown to be chemically competent chain elongation intermediates for the synthesis of two known aromatic polyketides. The *act* KS/CLF processed [^{14}C]acetyl-ACPs formed in ACPS reactions, lengthening the [^{14}C]acetyl group with malonyl-CoA-derived extender units to provide the usual C-16 polyketide products of the *act* PKS, SEK4, and SEK4b. It is notable that, although acetyl-CoA is not a preferred substrate for the *act* PKS in the presence of high concentrations of malonyl-CoA (Carreras et al., 1996; C. Carreras and C. Khosla, unpublished results), the acetyl groups of acetyl-ACPs are quantitatively incorporated into polyketides. The limiting factor in the amount of polyketide that can be made using this route seems to be the yield of acyl-ACP produced in ACPS reactions, which remains to be optimized for large scale acyl-ACP production.

The incorporation of acetyl groups attached to ACPs by ACPS into polyketide products demonstrates the viability of this un-natural pathway. This technique will be useful for the study of other putative acyl-ACP intermediates and is analogous to the use of NAC thioesters in the study of modular polyketide synthases (Yue et al., 198; Cane et al., 1987; Jacobsen et al., 1997; Pieper et al., 1995a,b). We also envision using ACP derivatives produced by ACPS to address other mechanistic questions regarding aromatic polyketide synthesis, such as the number of ACP molecules involved in the synthesis of a single polyketide chain. Finally, we note that this technology has potential for the generation of novel polyketides containing altered starter and extender units.

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