Purification and Properties of the *Streptomyces peucetius* DpsC β−Ketoacyl:Acyl Carrier Protein Synthase III that Specifies the Propionate-Starter Unit for Type II Polyketide Biosynthesis†

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ABSTRACT: Biosynthesis of the polyketide-derived carbon skeleton of daunorubicin (DNR) begins with propionate rather than acetate, which is the starter unit for most other aromatic polyketides. The *dpsC* gene has been implicated in specifying the unique propionate-starter unit, and it encodes a protein that is very similar to the *Escherichia coli* β-ketoacyl:acyl carrier protein (ACP) synthase III (FabH or KS III) enzyme of fatty acid biosynthesis. Purified DpsC was found to use propionyl-coenzyme A as substrate and to be acylated by propionate at the Ser-118 residue. DpsC exhibits KS III activity in catalyzing the condensation of propionyl-CoA and malonyl-ACP, and also functions as an acyltransferase in the transfer of propionate to an ACP. The DpsC enzyme has a high-substrate specificity, utilizing only propionyl-CoA, and not malonyl-CoA, 2-methylmalonyl-CoA or acetyl-CoA, as the starter unit of DNR biosynthesis.

The initial step in the biosynthesis of the daunorubicin (DNR)[1] polyketide involves the condensation of the malonate extender unit, as its malonyl-acyl carrier protein (ACP) derivative, with a specific starter unit made from propionyl-coenzyme A (CoA). In contrast, acetate via decarboxylation of malonyl-ACP is the starter unit in the formation of tetracenomycin (tcm) and actinorhodin (act), two well-characterized type II polyketide synthase (PKS)-derived natural products (*1*, *2*). The structure of the gene cluster responsible for the biosynthesis of DNR also differs from those containing the *tcm* or *act* genes. In the *act* (*3*) and *tcm* (*4*) gene clusters, the ACP gene of the type II PKS is immediately downstream of the genes for the two β-ketoacyl:ACP synthase (KS) subunits. In the *dps* gene cluster (*2*, *5*), the ACP gene is 6.8 kb upstream of the KS subunit genes, and *dpsC* and *dpsD*, two genes unique to the DNR cluster, are downstream of the latter genes. The deduced amino acid sequences of DpsC and DpsD are very similar to those of the *Escherichia coli* FabH (KS III) and FabD malonyl-CoA:ACP acyltransferase (MCAT) enzymes, respectively, and these findings have been used to suggest that DpsC and DpsD function like a KS III and MCAT in DNR biosynthesis (*2*, *5*).

Within *Streptomyces glaucescens* (*6*) and *Streptomyces coelicolor* (*7*), gene clusters that encode proteins with high-sequence similarity to the following components of the *E. coli* type II fatty acid synthase (FAS) have been discovered; a MCAT (FabD), ACP (FabC), KS III (FabH), and KS I (FabB). The *E. coli* KS I and II (FabF) enzymes catalyze the condensation of the extender unit, as its malonyl-ACP derivative, with the growing acyl chain to form the β-ketoacyl:ACP intermediate of fatty acid biosynthesis (*8*). KS III initiates the first condensation of malonyl-ACP with the starter unit acetyl-CoA to form acetoacetyl-ACP in the biosynthesis of straight-chain fatty acids (*8*−*10*).

The *S. glaucescens* FabH enzyme has been shown to function as a KS III, condensing different acyl-CoA substrates, as starter units, with malonyl-ACP (*11*). Branched-chain fatty acids are the predominate species produced in *Streptomyces* sp., and, through the use of precursor feeding studies, the amino acid catabolites isobutyryl-CoA, 2-methylbutyryl-CoA, and 3-methylbutyryl-CoA were found to be the biosynthetic-starter units of these fatty acids (*11*, *12*). Since thiolactomycin, a known type II FAS inhibitor, appeared to ameliorate the biosynthesis of the branched-chain fatty acids (*12*), it has been proposed that these fatty acids are made by the type II FASs encoded by the *S. glaucescens* and *S. coelicolor* genes. This idea is consistent with the fact that the *S. glaucescens* FabH enzyme is capable of utilizing four- and five-carbon acyl-CoA substrates as well as acetyl-CoA, suggesting that this FabH plays a role in choice of the starter units for biosynthesis of branched and straight-chain fatty acids (*11*).

Polyketide and fatty acid biosynthesis are comparable in terms of the enzymes involved, precursors used, and condensation reactions catalyzed (*1*, *13*). Reconstitution of the biosynthesis of actinorhodin and tetracenomycin precursors with purified enzymes has shown that a MCAT is

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[1] Abbreviations: AA, aklanonic acid; ACAT, acetyl-CoA:ACP acyltransferase; Act, actinorhodin; ACP, acylcarrier protein; CoA, coenzyme A.; DNR, daunorubicin; DTT, dithiothreitol; ES-MS, electrospray mass spectrometry; FAS, fatty acid synthase; HPLC, high-performance liquid chromatography; KS, ketoacyl:ACP synthase; kD, kilodaltons; MCAT, malonyl-CoA:ACP acyltransferase; PAGE, polyacrylamide gel electrophoresis; PKS, polyketide synthase; S., *Streptomyces*; TCA, trichloroacetic acid; Tcm, tetracenomycin; SDS, sodium dodecyl sulfate.

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required to produce malonyl-ACP as the extender unit (*14*, *15*), although there is some disagreement about the absolute need for the MCAT (*16*). The *act*-Orf1 and −Orf2 or TcmK and TcmL KS subunits carry out all the condensations of malonyl-ACP to the acyl-ACP formed in the previous step to produce the full-length polyketides containing 16 and 20 carbons, respectively (*14*, *15*). A KS III like activity was not found to be required in either system for the initial condensation reaction to yield acetoacetyl-ACP from acetyl-CoA and malonyl-ACP, indicating that the KS subunits are responsible for the first condensation reaction and, therefore, the choice of starter unit. By in vivo and in vitro biosynthetic studies using isotope-labeled precursors, we have recently proven that the function of the *dpsC* gene in DNR polyketide biosynthesis is to determine the choice of starter unit (*17*). Whether or not *dpsD* is also required has not been established, although it is known to be dispensable in vivo. Herein, we report the purification and characterization of DpsC, an enzyme that has KS III like activity and acyltransferase activity with high specificity for propionyl-CoA as the substrate.

EXPERIMENTAL PROCEDURES

Chemicals, Plasmid Constructs, and Bacteria Used. [2-^{14}C]-Malonyl-CoA was purchased from Amersham Life Science (Arlington Heights, IL) and [1-^{14}C]-propionyl-CoA from Moravek Biochemicals (Brea, CA). Acetyl-CoA, malonyl-CoA, propionyl-CoA, *E. coli* acyl carrier protein (ACP), and other commonly used chemicals were from Sigma (St. Louis, MO). SDS-PAGE gels (10−20%) were from BioRad (Hercules, CA). All FPLC columns were from Pharmacia Biotech (Piscataway, NJ). The *S. glaucescens fabD* (MCAT) expression plasmid was a gift from Kevin Reynolds of the University of Virginia Medical School (*11*). The *S. lividans* 1326 strain was obtained from David Hopwood (Norwich, UK).

Construction of dpsC and dpsG Expression Systems. For the construction of the *dpsC* expression plasmids, *Nde*I and *Eco*RI sites were introduced at the translational start codon and downstream of the translational stop codon of *dpsC*, respectively. The primers used for PCR were 5′-GGGAATTC*CATATG*AGCGTGCCGCAGGGGG-3′ and 5′-GGGAATTG*GAATTC*GGGCGCATCCATGACG-3′ (nucleotides in italics denote the *Nde*I and *Eco*RI sites, respectively). The PCR amplification reaction followed a procedure described earlier (*17*). The 1.2 kb PCR product was recovered by 0.8% agarose gel electrophoresis, digested with *Nde*I-*Eco*RI and ligated into the *Nde*I-*Eco*RI sites of plasmid T7SC (*18*). The resulting construct was digested with *Xba*I-*Hin*dIII and a resulting 1.2 kb fragment containing *dpsC* was ligated into the *ermE*p Streptomyces* expression plasmid pWHM1250 (*19*), which had been digested with *Xba*I-*Hin*dIII, to result in pWHM1017. To construct the expression plasmid for *dpsG*, a 500 bp fragment containing *dpsG* from pWHM555 (*20*) was cut out with *Pin*AI and ligated into the *Pin*AI site of LITMUS 28 (New England Biolabs, Beverly, MA). The fragment containing *dpsG* was excised with *Xba*I and *Hin*dIII from the latter plasmid and ligated into pWHM1250 resulting in pWHM1018.

Propionyl-CoA Binding Assay. To determine the amount of propionyl-CoA bound to the DpsC protein, the following assay was utilized. A typical assay solution included 5 μM [1-^{14}C]-propionyl-CoA (55 mCi/mmol), 2 mM dithiothreitol (DTT), 50 mM Tris-HCl (pH 7.2), and 5 μM DpsC enzyme. After 30 s at room temperature, the reaction was stopped with 300 μL of 20% trichloroacetic acid (TCA) and 100 μL bovine serum albumin (BSA) (10 mg/mL). Precipitated proteins were collected by microfuge centrifugation, and the pellets were washed with 10% TCA twice. The pellets were dissolved in 300 μL of 1 M Tris (pH 10.5), and 5 mL counting cocktail (Bio-Safe II, Research Products International Corp., Mount Prospect, IL) was added for liquid scintillation counting. The assay for MCAT activity was carried out as described previously (*15*).

Purification and Characterization of DpsC. Fresh *S. lividans* (pWHM1017) transformants, prepared by standard methods (*21*), were grown in R2YE medium containing thiostrepton as described previously (*15*). Culture harvesting, cell washing and lysis, and crude protein preparation followed the procedure reported earlier (*15*). Ammonium sulfate (40% of saturation) was added to the protein extract, and the solution was centrifuged at 25 420 × g, 4 °C for 20 min. Additional ammonium sulfate was added to give 60% of saturation to the supernatant, and the resulting mixture was centrifuged again at 25 420 × g, 4 °C for 20 min. The resulting pellet was dissolved in a buffer containing 50 mM Tris-HCl (pH 8.0), 2 mM DTT, and 5% glycerol (Buffer A). The enzyme solution (5 mL) was loaded on a Sephacryl S-200 FPLC column (Pharmacia Biotech, Piscataway, NJ) and eluted with the same buffer. The fractions with propionyl-CoA binding activity were collected and loaded on a Mono Q FPLC column. The column was washed with 20 mL buffer A and eluted with a 150 mL linear gradient of buffer A to 30% buffer B (buffer A containing 1 M NaCl). Fractions containing DpsC activity were pooled, and ammonium sulfate was added to 1.5 M. The solution was loaded to a phenyl superose FPLC column using buffer C (buffer A containing 1.5 M ammonium sulfate). The column was washed with 5 mL of buffer C at a flow rate of 0.25 mL/min, and protein was eluted by a 30 mL linear gradient of buffer C to buffer A. The active peak was collected, concentrated, and desalted into buffer A, using a Centricon 30 (Millipore, Bedford, MA) and stored at −80 °C.

Enzyme M_r Determination. Superose 12 gel filtration chromatography was used to determine the M_r of DpsC. After the column was washed, a 0.15 mL sample was loaded and the protein was eluted with buffer A at a flow rate 0.4 mL/min. A molecular weight standard curve was established following the method previously described (*15*), using blue dextran (M_r 2 000 000), alcohol dehydrogenase (M_r 150 000), BSA (M_r 66 000), carbonic anhydrase (29 000), cytochrome C (M_r 12 400), and tyrosine (M_r 181) standards (Sigma). SDS-PAGE gel electrophoresis of DpsC was done as described before (*15*). Propionyl-CoA bound-DpsC (1 μmol) was added to 100 μL hydroxylamine (0.5 M, pH 8.5) solution, which was incubated for 30 min at room temperature. The resulting solution was concentrated by a Centricon 30 and run on SDS-PAGE with the untreated propionyl-DpsC (1 μmol).

Mass Spectral Analysis of DpsC Labled with Propionate. Purified DpsC (15 μmol) and 20 μmol [1-^{14}C]-propionyl-CoA (0.05 mCi/mmol) in a 100 μL buffer solution (Tris-HCl, 50 mM, pH 7.2, 2 mM DTT) were incubated at room

temperature for 1 min. A Centricon 30 was used to remove the salt and unbound propionyl-CoA and to concentrate propionyl-DpsC into water. A 15 μL solution containing the labeled DpsC was analyzed by electrospray mass spectrometry (ES-MS)/HPLC.

To obtain the sequence of the propionyl binding fragment, the propionylated DpsC protein was subjected to trypsin digestion. The reaction (100 μL) consisted of 2 μg trypsin, 50 mM ammonium carbonate, pH 8.5, and 50 μg of propionyl-DpsC and was incubated overnight at 37 °C. The peptide fragments were separated on a C-18 reverse phase HPLC column (Protein and Peptide C_{18}, Vydac) with a Waters 484 variable wavelength absorbency detector and a Radiomatic Flo-One/Beta A-515 radio-chromatography detector. A gradient of water/acetonitrile/trifluoric acetic acid (90:10:0.01 to 0:100:0.01, vol/vol) in 150 min at a flow rate of 0.5 mL/min was used to elute the peptide fragments from the column. The radioactive peak was collected, dried under vacuum, and analyzed by ES-MS/HPLC.

Assays for KS III and Acyltransferase Activity. The 100 μL acyltransferase activity assay solution contained 50 μM [1-^{14}C]-propionyl-CoA (2 mCi/mmol), 50 μM *E. coli* ACP, 0.5 μM DpsC, 2 mM DTT, and 50 mM Tris-HCl, pH 7.2. The reaction was initiated by addition of DpsC, incubated at room temperature for 2 min, and stopped with addition of 100 μL BSA (10 mg/mL) and 300 μL TCA (20%, w/v). Protein precipitation, pellet washing, and liquid scintillation counting were done as described above for the propionyl-CoA:DpsC binding assay. To assay DpsC acyltransferase activity with DpsG, ACP holoenzyme from *S. lividans* was assayed and prepared. The culture conditions for the strain of *S. lividans* containing plasmid pWHM1018, cell harvesting, protein extraction, ammonium sulfate, and acetic acid precipitation were done as described for the TcmM ACP (*22*). The pellets from ammonium sulfate and acetic acid precipitation were combined and dissolved in buffer A, loaded on a Sephacryl S-200 column, and eluted as described for DpsC purification. The fractions containing ACP activity were loaded on a Mono Q FPLC column and eluted with a 150 mL linear gradient of buffer A to 40% buffer B. Ammonium sulfate (1.5 M) was added to the fractions with ACP activity, and the solution was loaded on a phenyl superose column. The activity was eluted by a 30 mL linear gradient of buffer C to buffer A. Fractions with ACP activity were pooled, desalted into buffer A by using Centricon 10, and used in assays to test the acyltransferase activity of DpsC.

To assay for KS III activity, malonyl-ACP was required and produced as follows. Reactions contained 100 μM *E. coli* AcpP ACP, 300 μM malonyl-CoA, 2 μM *S. glaucescens* FabD MCAT, 2 mM DTT, and 50 mM Tris-HCl, pH 7.2 in a total volume of 100 μL. The partially purified DpsG ACP was treated identically, but its concentration was unknown. The reactions were incubated at ambient temperature for 10 min after which 50 μL was removed for the KS III reaction. The KS III reaction (100 μL) contained 50 μM [1-^{14}C]-propionyl-CoA (2 mCi/mmol), 50 μM malonyl-ACP, 0.5 μM DpsC, 2mM DTT, and 50 mM Tris-HCl, pH 7.2, and was initiated by addition of DpsC and terminated after 1 min at room temperature by addition of 100 μL BSA (10 mg/mL) plus 300 μL 20% TCA. The precipitated protein was collected by centrifugation and the pellet was washed twice with 10% TCA. The pellet was dissolved in 300 μL 1 M Tris base solution and transferred to 5 mL of scintillation cocktail for counting. The M_r of the modified ACP was determined by ES-MS/HPLC, using samples from the KS III assay and the acyltransferase assay. A portion of the samples from the KS III assay, the acyltransferase assay, and the DpsC propionyl-CoA binding assay was also separated on SDS-PAGE to visualize the M_r of the proteins containing a [1-^{14}C]-propionyl group. The [1-^{14}C]-propionyl-CoA was separated from the assay products by a Centricon 10 before loading on the gel.

Table 1: Purification of DpsC

purification steps	protein amount (mg)
crude cell extract	3500
(NH4)2SO4	650
sephacryl S-200	85
mono Q	4
phenyl superose	1.2

RESULTS AND DISCUSSION

Since we have established that the *dpsC* gene plays a dominant role in starter unit selection, through studies of the DNR PKS in vivo and in vitro (*17*), purification of the DpsC protein was undertaken. The *dpsC* gene was cloned into a vector for *Streptomyces* expression as described in the Experimental Procedures. The crude protein extract of *S. lividans* containing pWHM1017 (see Experimental Procedures) was found to bind [1-^{14}C]-propionyl-CoA. Utilizing the self-acylation activity as an assay, DpsC was purified through ammonium sulfate precipitation, S-200 gel filtration, Mono Q ion exchange, and phenyl superose chromatographic steps (Table 1). The acylation activity was checked in each step of the ammonium sulfate precipitation where most of the activity was found in the precipitate of 40 to 60% saturation. The crude precipitate was separated on an S-200 sephacryl gel filtration column and the activity was found to elute in the lower molecular weight fractions. The pooled fractions containing propionyl-CoA acylation activity were chromatographed on a Mono Q ion exchange column with the activity being eluted at a very low concentration of salt (0.2 M NaCl). In the final purification step, the acylation activity was eluted from a phenyl superose column as one discrete peak (Figure 1A, lane 2). The M_r of DpsC was estimated using gel filtration chromatography, to be 38 kDa (Figure 2), which is in agreement with the M_r calculated from the predicted sequence of DpsC (*2*). Interestingly, this result suggests that the active form of DpsC is monomeric, as compared to the homodimeric structures of *E. coli* FabH and FabD, proteins that catalyze similar reactions in fatty acid biosynthesis (*8*).

Purified DpsC containing a [1-^{14}C]-propionyl group showed a single band at the position of DpsC (Figure 1B, lane 1), indicating that the propionate group had remained covalently attached to DpsC during the electrophoresis on a denaturing gel. Furthermore, propionylated DpsC samples boiled prior to SDS-PAGE contained approximately the same amount of ^{14}C-radioactivity as the unboiled control (Figure 1B, lane 2), and treatment of propionylated DpsC with basic hydroxylamine, an agent known to cleave thioester bonds, failed to diminish the amount of ^{14}C associated with DpsC (Figure 1B, lane 3). Together, these observations established that the

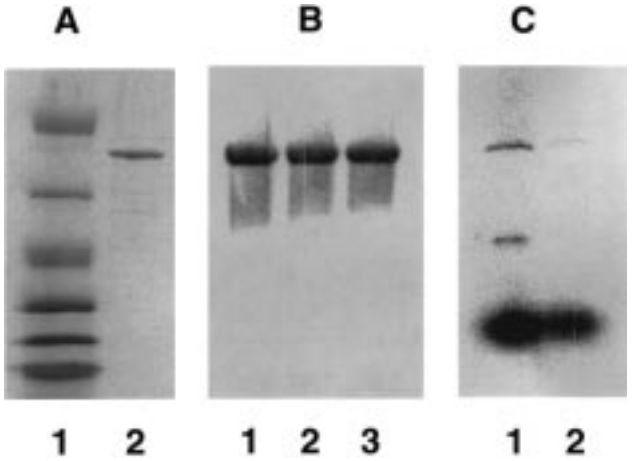

FIGURE 1: SDS-PAGE analysis of DpsC and *E. coli* ACP. (A) Purified DpsC stained with coomassie blue. Lane 1, molecular weight markers; lane 2, purified DpsC. (B) Autoradiogram of SDS-PAGE of the [1-^{14}C]-propionylated DpsC. Lane 1, untreated; lane 2, boiled for 3 min; lane 3, treated with basic hydroxylamine. (C) Autoradiogram of SDS-PAGE showing transfer of [1-^{14}C]-propionate from propionyl-CoA to *E. coli* holo-ACP and malonylated-ACP. Lane 1, transfer to holo-ACP; lane 2, transfer to malonyl-ACP.

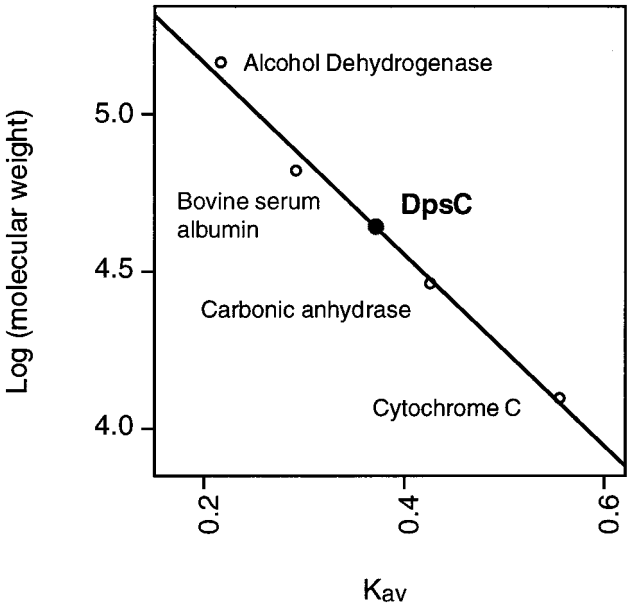

FIGURE 2: Estimation of the molecular weight of DpsC by gel filtration chromatography. $K_p = (V_e - V_o)/(V_t - V_o)$. V_o and V_t were determined by using blue dextran and tyrosine, respectively.

propionyl group was associated with DpsC through a covalent bond. To determine the precise nature of propionate attachment, the protein samples were analyzed by ES-MS/HPLC. The estimated average M_r for the ^{14}C-bound protein was found to be 37 189, a mass very close to the estimated average M_r of DpsC (37 132) that has been modified by the addition of a propionyl group. [1-^{14}C]-Propionylated DpsC was digested with trypsin, and the resulting peptide fragments were separated on a C-18 reverse phase HPLC column. The fraction containing radioactivity was collected and again analyzed via ES-MS. Within this fraction, four peptides of differing M_r were detected, with one of the fragments displaying a M_r that differed from that calculated on the basis of the DpsC sequence by 56, the molecular mass of a propionyl group. MS sequence analysis of this fragment

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          1                                                  50
DpsC      MSvpqGAPGD LY..VaGcGv WLPPpVtTeq aLA...Aghc DRrLasStrm
DpsC/C5   MStLpGAPGD LY..VaGcGv WLPPpVtTdq aLA...Aghc DRrLasStrm
FrnI      ...MlGtrpv vhsRllGvGg YrPrrsvdNa eLcaTVAstp EWIetRSGIr
SgFabH    MSkikpAkGa pYaRIlGvGg YrPtrVvpNe vileTIdssd EWIrsRSGIq
EcFabH    ..........MYtkIiGtGs YLPeqVrTNa dLekmVdtsd EWIvtRtGIr

          51                                                 100
DpsC      LsvavADkET pAEMaAlAAq tAlDRsGVaP AhVdLVLhAS LYfQgHhLwA
DpsC/C5   LsvavADkET pAEMaAsAAr tAVDRsGVpP ArIvLVLhAS LYfQgHhLwA
FrnI      aRgfAApdET lrfMgraAAe kAlaRAGVlP dgIdLVLVAS M.srleqtPp
SgFabH    tRhwAnDeET vAaMsiEAsg kAIadAGIta AQVgaViVst v.thfkqtPA
EcFabH    eRhiAApnET vstMgfEAAt rAIEmAGIek dQIgLIvVAt t.satHaFPs

          101                 ********** ****             150
DpsC      PsSYVQrVav GNRCPAMEVr qv**S**nGgMaAL ELARAYlLAa pdRvAALItt
DpsC/C5   PASYVQrVal GNRCPAMEVr qv**S**nGgMaAL ELARAYlLAa pdRtAALVtt
FrnI      lAvllaedLG araaagLDVs ga**C**AGFchAL aLAsdaVrAG saRh.vLVVG
SgFabH    vAteIadkLG tNkaaAFDIs ag**C**AGFgygL tLAkgmIveG saeY.vLVIG
EcFabH    aAcqIQsmLG ikgCPAFDVa aa**C**AGFtyAL svAdqYVksG avkY.ALVVG
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FIGURE 3: Protein sequence comparisons of DpsC (*2*), DpsC/C5 (*5*), FrnI, *S. glaucescens* FabH (*6*) and *E. coli* FabH (*9*) by the PILEUP method (*31*). [DpsC homlogues have also been found in the *red* (GenBank accession number AL021409) and two other (www.sanger.ac.uk/Projects/S_coelicolor/) gene clusters in the *S. coelicolor* genome sequencing project.] Only the first 150 residues of each protein are shown. The active site residues are in bold type. Region sequenced by mass spectrometry is indicated by asterisks.

showed that it contained the amino acid sequence from residue 116 to 129 in DpsC and that the propionyl group was bound to serine-118. Interestingly, KS III enzymes from *E. coli* and *Streptomyces* all have a cysteine at this position that is believed to bind the acyl group via a thioester bond (Figure 3). This has been confirmed for the *E. coli* enzyme; its Cys-112-Ser mutant also is acylated at the serine residue but exhibits 10^3- to 10^4-fold less KS III activity than the wild-type enzyme.[2]

The ability of DpsC to be acylated by propionate suggests that the protein could function similar to a KS III in fatty acid biosynthesis, catalyzing the first condensation of the propionate-starter unit with malonyl-ACP to create β-ketovalerate bound to the phosphopantetheine prosthetic group of holo-ACP. To test this hypothesis, DpsG, the PKS-specific ACP encoded by a DNR biosynthesis gene (*2*, *5*), was expressed in *S. lividans* as described in the Experimental Procedures. The protein fraction containing DpsG was enriched by ammonium sulfate precipitation, Mono Q and phenyl superose FPLC. The resulting protein sample was assayed by SDS-PAGE, which revealed several mirror protein bands on the gel plus a major one which had the mobility expected for DpsG. DpsC was found to be required to transfer the propionyl group from [1-^{14}C]-propionyl-CoA to DpsG (data not shown) using this protein preparation, suggesting that DpsC could function as an acyltransferase. (This conclusion does not exclude the possibility that other proteins with MCAT activity contributed to the result.) To further examine the functional activity of DpsC, purified *E. coli* AcpP ACP was utilized in the MCAT assay (Table 2). With the *E. coli* ACP, DpsC exhibited two types of activity;

[2] Charles Rock, personal communication.

Table 2: Catalytic Activity of DpsC

	relative activity	
	ACP (acyltransferase)	malonyl-ACP (KS III)
propionyl-CoA	100	100
malonyl-CoA	<1	<1
2-Methylmalonyl-CoA	<1	<1
acetyl CoA	<1	<1

these included the transfer of the propionyl group from propionyl-CoA to the AcpP protein (Table 2 and Figure 1C, lane 1), functioning as an acyltransferase, and the condensation of the propionyl-CoA to malonyl-AcpP, a KS III like activity (Table 2 and Figure 1C, lane 2). The DpsC-catalyzed modifications of *E. coli* holo-ACP were verified by ES-MS/HPLC analysis. The results showed that the estimated average M_r of the propionyl-ACP was 8906 and that of β-ketovaleryl-ACP was 8948, values that are in agreement with theoretical calculations for these acylated proteins (estimated average M_r for *E. coli* holo-ACP is 8850). The acyltransferase activity appears to be as efficient as the KS III like activity with *E. coli* ACP (Table 2, Figure 1C). These activities were highly specific in terms of the substrate used; DpsC could not use malonyl-CoA, 2-methylmalonyl-CoA or acetyl-CoA in either the acyltransferase or KS III assays (Table 2).

The data resulting from the above experiments show that the enzyme activity of DpsC resembles that of KS III in fatty acid biosynthesis. The *E. coli* KS III enzyme catalyzes condensation of the acetyl-CoA-starter unit with the first chain extender unit, malonyl-AcpP, in the formation of acetoacetyl-AcpP. This enzyme also has acetyl-CoA:ACP acyltransferase (ACAT) activity, which results in the production of acetyl-ACP. However, the specific ACAT activity of *E. coli* FabH is only 0.5% of its KS III activity (*9*). The *S. glaucescens fabH* gene encodes a protein with both ACAT and KS III activities (*11*). The ACAT activity is approximately 12% of the KS III activity with various substrates, such as acetyl-CoA, butyryl-CoA, and isobutyryl-CoA. This FabH is believed to use these substrates as starter units and to form straight and branched chain fatty acids (*11*). DpsC, on the other hand, has a high-substrate specificity for propionyl-CoA as its substrate and, thus, habors no significant ACAT activity.

Most gene clusters that govern the biosynthesis of aromatic polyketides, via type II PKSs, do not contain homologues of the *S. peucetius* (*2*) or *Streptomyces* sp. strain C5 (*5*) *dpsC* or *dpsD* genes (*1*, *23*, *24*). This may stem from the fact that acetate is the predominant starter unit used in the majority of pathways studied to date. For instance, in the case of the 16- and 20-carbon polyketides, SEK4, SEK4b (*14*), and Tcm F2 (*15*), which are shunt products and precursors of actinorhodin and Tcm C biosynthesis, respectively, the PKSs reconstituted from purified KS, holo-ACP, and MCAT enzymes use only malonyl-CoA as the chain starter and extender unit to produce the polyketide products in vitro. It appears that the acetate-starter unit arises by decarboxylation of the malonyl-ACP or similar intermediate, although the available data do not exclude the possible use of acetyl-CoA as the starter unit in vivo. The latter possibility would be consistent with isotope labeling data reported over the past 25 years for the biosynthesis of several bacterial aromatic

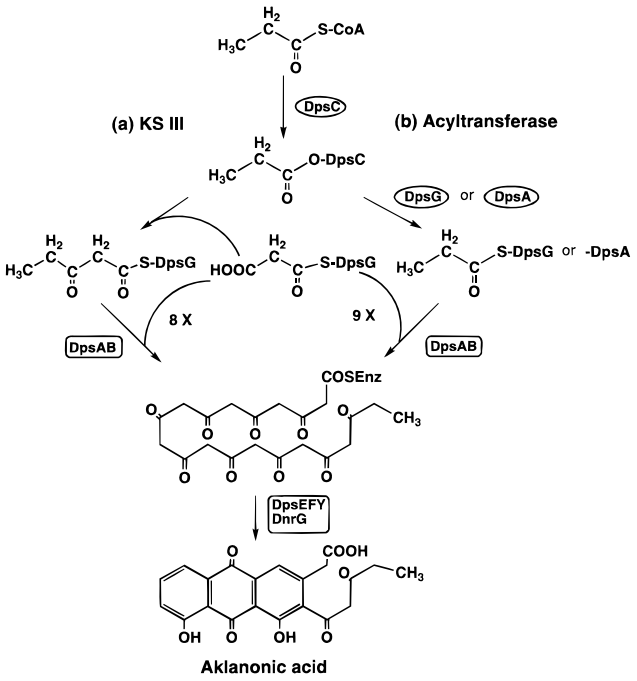

FIGURE 4: Schematic representation of possible DpsC functions in the biosynthesis of aklanonic acid, a key intermediate of the DNR pathway. Functioning (a) as a KS III and (b) as an acyltransferase.

polyketides (*25*). No KS III nor ACAT activity has been reported for any of these enzymes, although this may only mean that they were not assayed for such activity in the past. DNR biosynthesis stands out as a special case since its polyketide skeleton is clearly made from a propionate-starter unit, both in vivo and in vitro (*17*), and, therefore, it is not surprising that the DpsC enzyme is needed to specify the use of propionyl-CoA. Frenolicin, an 18-carbon aromatic polyketide (*23*), may use butyrate as the starter since its gene cluster contains *dpsC* and *dpsD* homologues (GenBank, accession number AF058302). Oxytetracycline biosynthesis uses a malonamide-starter unit of unknown origin. Its gene cluster does not contain a *dpsC* homolog, but has a *dpsD* homologue and a carboxy-CoA ligase gene that together may govern how this novel starter unit is used (*26*).

At the present time, we cannot distinguish whether DpsC functions in DNR biosynthesis primarily as a ketosynthase or acyltransferase, or has both activities. Since *dpsD*, the gene next to *dpsC* in the DNR cluster, is likely to encode a MCAT (*2*, *5*, *17*, *27*) that is specific for the DpsG ACP and malonyl-CoA to make malonyl-DpsG, DpsC could function primarily as a KS III like enzyme to make β-ketovaleryl-DpsG. This product would then be used by the DpsA and DpsB KS subunits to synthesize a full-length 21-carbon polyketide, using eight-extender units in their malonyl-DpsG forms (Figure 4(a)). On the other hand, since DpsC can also transfer propionyl-CoA to ACP to form propionyl-ACP, the KS subunits alternatively might catalyze both the first and subsequent condensation reactions to make the full-length polyketide (Figure 4). If the number of condensation steps is determined only by the KS subunits, DpsC would act only as an acyltransferase. This idea would include the possibility that DpsC transfers propionate to the DpsA KS subunit (Figure 4(b)) so that β-ketovaleryl-DpsG could be formed by a condensation reaction between propionyl-DpsA and

malonyl-DpsG. Since it has been reported that 20-carbon polyketides can be made by the DNR PKS in the absence of DpsC in vivo and in vitro (*17*, *28*, *29*) and that DpsA and DpsB form Tcm F2 when combined with the TcmM ACP and TcmN cyclase (*17*, *28*), the Dps KS subunits appear to catalyze nine condensation reactions, the same number of condensation steps as in Tcm F2 biosynthesis. DpsC thus is likely to function only as a acyltransferase in the formation of propionyl-DpsG or -DpsA. However, the current information about type II PKSs is consistent with the belief that the number of condensation reactions is controlled by the full complex of KS, ACP, MCAT, cyclase, and ketoreductase enzymes, instead of by the KS or KS and ACP enzymes alone (*23*, *30*). Until this question is examined in vitro using the fully panopoly of the DNR PKS enzymes, we cannot know with certainty which function of DpsC is most important in DNR biosynthesis, nor determine whether the DpsD MCAT is a critical component of this process.

NOTE ADDED IN PROOF

An intriguing question arises when the two roles of DpsC shown in Figure 4 are considered in relation to the ability of the DpsA and DpsB KS subunits to catalyze the decarboxylation of malonyl-ACP to acetyl-ACP when combined with the TcmM ACP and TcmN cyclase enzymes or in the absence of DpsC. In the native Dps PKS, this decarboxylation reaction must be suppressed to avoid formation of the 20-carbon desmethyl AA from an acetate-starter unit. DpsC thus may produce propionyl-DpsG at a much faster rate than acetyl-DpsG can be formed, or the propionyl-DpsA formed from propionyl-DpsG may inhibit the utilization of acetyl-DpsG as a source of the starter unit. In either case, it is unlikely that the propionate-starter unit can arise by decarboxylation of 2-methylmalonyl-DpsG.

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