Propionyl-CoA carboxylase from *Streptomyces coelicolor* A3(2): cloning of the gene encoding the biotin-containing subunit

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In *Streptomyces coelicolor* A3(2), polyketides are made from malonyl-CoA, which is presumed to be derived from acetyl-CoA by the action of acetyl-CoA carboxylase (ACC). No ACC activity was found in cell-free extracts of *S. coelicolor*. However, propionyl-CoA carboxylase (PCC) activity was detected at substantial levels. Fixation of CO₂ by ACC and PCC occurs by covalent bonding of CO₂ to a biotin-containing protein. Most bacteria have a single small biotinylated protein of approximately 22 kDa, but *S. coelicolor* contains three larger biotin-containing proteins (approximately 145, 88 and 70 kDa). To determine which biotinylated protein was associated with PCC activity, the enzyme was purified and shown to comprise an α subunit (biotin-containing) of 88 kDa and a β subunit of 66 kDa. The N-terminal sequences of these proteins were determined and, using an oligonucleotide probe, the gene for the α subunit (*pccA*) was cloned.

Keywords: *Streptomyces coelicolor* A3(2), acetyl-CoA carboxylase, propionyl-CoA carboxylase, biotinylated proteins

INTRODUCTION

Bacteria of the genus *Streptomyces* have been studied widely due to their relatively complex life cycle and their ability to produce a variety of medically and/or industrially useful secondary metabolites. *Streptomyces coelicolor* A3(2) has been used as a model species in these studies and is genetically well-characterized (Kieser *et al.*, 1992). This micro-organism produces at least four secondary metabolites (Hopwood *et al.*, 1994), at least two of which (in common with a large number of medically important antibiotics) are wholly or partly polyketide-derived (Gorst-Allman *et al.*, 1981; Wasserman *et al.*, 1976). The spore pigment is also a polyketide, whose biosynthesis is encoded by the *whiE* gene cluster (Davis & Chater, 1990). Polyketide biosynthesis occurs by a series of reactions analogous to those by which fatty acids are made, so

malonyl-CoA is the predicted substrate for all of the condensation reactions which yield these polyketides. Whereas the genes for biosynthesis of secondary metabolites and their regulation have been studied in considerable depth, primary metabolism (which must supply the building blocks for polyketide biosynthesis) is still poorly understood. Information gained on the enzymes involved in the supply of precursors should enable the manipulation of their supply, and the alteration of the types and yields of useful secondary metabolites.

In our previous work, we have shown that phosphoenolpyruvate carboxylase (PEPC) is the sole anaplerotic enzyme present in extracts of *S. coelicolor* when grown on glucose, and that the specific activity of the enzyme rose during polyketide biosynthesis (Bramwell *et al.*, 1993). This anaplerotic enzyme acts to replenish intermediates of the tricarboxylic acid cycle, which are removed for the biosynthesis of other metabolites. For *S. aureofaciens*, which produces the polyketide tetracycline, Behal *et al.* (1977) have reported the characterization of an enzyme, oxaloacetate (OAA) dehydrogenase, which would convert OAA (the product of the PEPC-catalysed reaction) into malonyl-CoA. Our initial working hypothesis was that malonyl-CoA was made from PEP by these two sequential reactions and the enhanced activity of PEPC

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Abbreviations: ACC, acetyl-CoA carboxylase; BCCP, biotin carboxyl carrier protein; PCC, propionyl-CoA carboxylase; PEP (PEPC), phosphoenolpyruvate (carboxylase).

Fig. 1. Overall scheme of the reaction catalysed by ACC. The biotinylated subunit (E-Biotin) is recycled as shown by the dashed arrow. The reaction scheme for PCC is similar, except that propionyl-CoA is used as substrate for the carboxyl transferase step and methylmalonyl-CoA is produced.

was to provide carbon flux for polyketide biosynthesis. However, we were unable to find any evidence for OAA dehydrogenase in *S. coelicolor*. In all species which have been studied so far (Bloch & Vance, 1977; Harwood, 1988), the malonyl-CoA used in the biosynthesis of fatty acids is produced by carboxylation of acetyl-CoA (Fig. 1), and it was considered likely therefore that acetyl-CoA carboxylase (ACC) activity should also be important in the production of precursors of polyketides.

ACC has been purified from a number of species and in some organisms this enzyme also catalyses the carboxylation of propionyl-CoA to methylmalonyl-CoA (Fig. 1; Erfle, 1973; Hunaiti & Kolattukudy, 1982; Meyer & Meyer, 1978). We detected a high activity of propionyl-CoA carboxylase (PCC) in cell-free extracts of *S. coelicolor*, suggesting that this enzyme plays a major role in the metabolism of this organism. However, we failed to detect ACC activity. As a preliminary step in the investigation of the precise role of the enzyme in *S. coelicolor*, we have purified PCC, subjected the protein to N-terminal sequencing and cloned the gene encoding one of the subunits. The potential roles of PCC in the growth of *S. coelicolor* and polyketide biosynthesis are discussed.

METHODS

Materials. Acetyl-CoA, propionyl-CoA, 4-chloro-1-naphthol and Avidin were obtained from Sigma, ATP was from Boehringer, streptavidin-horseradish peroxidase complex, NaH¹⁴CO₃ (0.1 mCi mmol⁻¹) and membranes were from Amersham. DEAE-Sephacel (fast flow) and CNBr-activated Sepharose were from Pharmacia. Restriction enzymes were obtained from Gibco-BRL, sequencing reagents were from USB, T7 DNA polymerase for sequencing was from Pharmacia and DNA ligase was from Promega.

Avidin was coupled to CNBr-activated Sepharose as described by the manufacturer. The Avidin bound to the column was converted to the low-affinity monomeric form by denaturation with guanidinium HCl and high-affinity binding sites were saturated with biotin as described by Henrikson *et al.* (1979).

Growth of cells and preparation of cell-free extracts. *S. coelicolor* A3(2), strain 1147 (SCP1⁺, SCP2⁺; Hobbs *et al.*, 1990)

was grown on YEME (Hopwood *et al.*, 1985). Cultures were inoculated with fresh spores, grown, harvested and disrupted as described previously (Bramwell *et al.*, 1993). PCC was purified from 32 g wet weight of cells.

Enzyme assays. Four assays for ACC activity were attempted. These were based on modifications of the procedures used to detect the mammalian (Holland *et al.*, 1984), *Escherichia coli* (Alberts & Vagelos, 1968) and plant (Nikolau *et al.*, 1981) ACCs and that used to detect PCC from *S. erythraea* (Hunaiti & Kolattukudy, 1982). The reagents for the assays, each mixed in a microfuge tube, were as follows: (a) 8.3 µmol Tris/HCl, pH 8.0, 0.1 mg BSA, 330 nmol ATP, 830 nmol tri-sodium citrate, 990 nmol MgCl₂, 4 µmol NaH¹⁴CO₃ and 25 nmol acetyl-CoA; (b) 7 µmol imidazole/HCl, pH 6.9, 44 nmol ATP, 44 nmol MnCl₂, 1.5 µmol NaH¹⁴CO₃ and 30 nmol acetyl-CoA; (c) 2 µmol Tris/HCl, pH 7.5, 100 nmol dithiothreitol, 5 µmol KCl, 100 nmol ATP, 250 nmol MgCl₂, 3 µmol NaH¹⁴CO₃ and 30 nmol acetyl-CoA; (d) assay mixture for PCC as described by Hunaiti & Kolattukudy (1982) (see below), but with 200 nmol acetyl-CoA as substrate in place of propionyl-CoA. Protein-containing extract (20 µl) was added to give a total volume of 100 µl and the mixtures were incubated at 30 °C for 15 min. The reactions were stopped by addition of 10 µl 6 M HCl and 50 µl aliquots dried onto 2.5 cm diameter Whatman No. 1 filter paper discs with the aid of a hair dryer. The discs were placed in 3 ml scintillation vials with 0.5 ml water and 2.5 ml Ecocint and ¹⁴C-radioactivity was determined for either 1 or 10 min.

PCC activity was determined by the method of Hunaiti & Kolattukudy (1982). The reaction contained 8 µmol potassium phosphate, pH 8.0, 0.3 mg BSA, 300 nmol ATP, 500 nmol MgCl₂, 3 µmol NaH¹⁴CO₃, 200 nmol propionyl-CoA and 30 µl protein-containing extract in a total volume of 126 µl. The reactions were incubated at 30 °C, stopped by acidification and counted as above. One unit of enzyme activity catalysed the incorporation of 1 µmol ¹⁴C into acid-stable products min⁻¹. Protein concentrations were determined by the method of Bradford (1976).

Detection of biotinylated proteins. Extracts were analysed for biotinylated proteins by a modification of the Western blotting procedure described by Nikolau *et al.* (1985). After separation by SDS-PAGE (Laemmli, 1970), proteins were electroblotted on to nitrocellulose membrane in 20 mM Tris, 150 mM glycine (pH 8.3), 20% (v/v) methanol, at 300 mA for 3 h. Filters were blocked in PBS containing 0.25% (w/v) gelatin and incubated with a 1/300 dilution of HRP-streptavidin conjugate for 30 min at room temperature. After washing in blocking buffer, HRP which was bound to the filter via the streptavidin-biotin interaction was visualized by incubating with 50 ml 10 mM Tris/HCl, pH 7.2, 20% methanol containing 150 µl 4% (v/v) H₂O₂ and 30 mg 4-chloro-1-naphthol.

Purification of PCC. All procedures were carried out at 4 °C, except for FPLC which was at room temperature.

(NH₄)₂SO₄ fractionation. The cell-free extract was adjusted to 30% (w/v) saturation with (NH₄)₂SO₄ and stirred for 30 min. The supernatant obtained after centrifugation at 27000 g for 15 min was taken to 60% saturation by addition of further (NH₄)₂SO₄, stirred for 30 min and centrifuged at 27000 g for 15 min. The pellet was resuspended in a minimal volume of 100 mM potassium phosphate buffer, pH 7.0, containing 10% (v/v) glycerol, 5 mM EDTA, 1 mM benzamidine and 0.4 mM dithiothreitol (buffer A). The (NH₄)₂SO₄ pellet was desalted on a Sephadex G25 column (200 ml bed volume) at a flow rate of 100 ml h⁻¹. Fractions eluting in the void volume which gave a positive reading at A₂₈₀ were pooled and loaded onto the DEAE-Sephacel column.

DEAE-Sephacel chromatography. A DEAE-Sephacel column (20 ml bed volume) was equilibrated in buffer A at a flow rate of 30 ml h⁻¹. The desalted (NH₄)₂SO₄ pellet was loaded onto the column at the same flow rate and the column was washed until the *A*₂₈₀ of the eluant was equal to that of buffer A. Proteins were eluted from the column with a linear gradient of 0–1 M KCl in a volume of 300 ml. Fractions (5 ml) were assayed for PCC activity.

Avidin-Sepharose chromatography. Fractions that eluted between 0.1 and 0.3 M KCl from the DEAE-Sephacel column contained PCC activity. These were pooled and dialysed overnight against 100 mM Tris/HCl, pH 7.5, containing 10% glycerol, 5 mM EDTA (buffer B). An Avidin-Sepharose column (5 ml bed volume) was equilibrated in buffer B and loaded with dialysate from the DEAE-Sephacel column at a flow rate of 30 ml h⁻¹. The column was washed in buffer B to remove unbound material, and biotin-containing proteins were eluted isocratically in buffer B containing 0.2 mM biotin at the same flow rate. Fractions (2.5 ml) were collected and assayed for PCC activity.

Anion-exchange chromatography. Fractions from the Avidin-Sepharose column were pooled and loaded onto a MonoQ column which had been equilibrated in buffer B. Unbound material was removed by washing in buffer B and bound proteins were eluted with the following gradient in buffer B: 0–0.214 M NaCl over 6.42 ml, 0.214 M NaCl for 2 ml, 0.214–0.299 M NaCl over 2.55 ml, 0.299 M NaCl for 2 ml and 0.299–1 M NaCl over 21 ml. Fractions having *A*₂₈₀ above that of buffer B were assayed for PCC activity.

SDS-PAGE. The fractions from the MonoQ column containing PCC activity were assessed for purity by SDS-PAGE and proteins were visualized by staining with silver (Porro *et al.*, 1982).

Protein sequencing. Proteins were electroblotted onto ProBlott membranes (Applied Biosystems) by a modification of the procedure described by Matsudaira (1987). Fractions 25 and 26 from the MonoQ column were pooled and run on SDS-PAGE gels in Tris/Tricine buffer. A solution of 0.1 M Tris/0.1 M Tricine/0.1% SDS, pH 8.25, was placed in the upper electrophoresis tank and 0.1 M Tris/HCl, pH 8.9 was placed in the lower chamber. Before loading the samples, 8 mM glutathione was added to the upper reservoir and the gel was pre-run at 6 mA constant current for 1 h. After pre-running, buffers from the upper and lower reservoirs were replaced and 50 mM sodium thioglycolate was added to the upper tank. Samples were loaded and the gel was run at 100 V.

Proteins were transferred to a ProBlott membrane in 3-[cyclohexylamino]-1-propane sulphonic acid buffer, pH 11.0, containing 10% methanol, at 250 mA for 75 min. The membrane was rinsed in water before staining with 0.1% amido black in 40% methanol/1% acetic acid until bands were visible. Background staining was reduced by washing in water and the filter was dried in air. Appropriate bands were excised and loaded directly onto an Applied Biosystems model 477A gas-phase sequencer. Amino acid thiophydantoin were detected using an Applied Biosystems model 120A on-line analyser.

Molecular biological techniques. The procedures involved in the cloning of DNA were carried out as described by Sambrook *et al.* (1989). Digests of genomic DNA were transferred to Hybond-N filters in alkali transfer buffer as described by the manufacturers. DNA fragments were purified after excision from agarose gels by centrifugation through Spin-X tubes supplied by CoStar. Single-stranded template was sequenced at 37 °C in the presence of 7-deazanucleotide triphosphates to sequence through regions of dG+dC-rich DNA. Sequences

were compiled and analysed using programs of the University of Wisconsin Genetics Computer Group (Devereux *et al.*, 1984).

Cloning of the putative *pccA* gene. Total genomic DNA from *S. coelicolor* was prepared as described by Hopwood *et al.* (1985). DNA was digested under appropriate conditions (as defined by BRL) with a variety of restriction endonucleases. Each digest was loaded onto a 1 cm thick 0.8% agarose gel containing ethidium bromide (0.5 µg ml⁻¹). Electrophoresis was carried out overnight at 0.8 mA cm⁻¹ and DNA was transferred from the gel to Hybond-N membranes. Fragments containing a sequence which would encode the 88 kDa subunit of *S. coelicolor* PCC were identified by hybridization to an oligonucleotide probe designed using the N-terminal sequence of the protein. Hybridization was at 55 °C in 5 × SSC (Sambrook *et al.*, 1989) and washes were at 60 °C in 1 × SSC. DNA hybridizing to the oligonucleotide was excised from a separate gel and cloned into *E. coli* TG1 in the vector pUC18 (Sambrook *et al.*, 1989).

RESULTS AND DISCUSSION

Biotinylated proteins from *S. coelicolor*

ACC is a biotin-dependent enzyme whose subunit structure differs between species. In many bacteria, the three components [biotin carboxylase, biotin carboxyl carrier protein (BCCP) and carboxyl transferase] required to catalyse the overall reaction (Fig. 1) are individual proteins encoded by different genes, whereas the vertebrate and yeast ACCs are multifunctional polypeptides encoded by a single gene (Al-Feel *et al.*, 1992; Li & Cronan, 1992a, b; Lopez-Casillas *et al.*, 1988). The functional domains of the vertebrate polypeptide have been identified by comparing sequences of the deduced gene products of the vertebrate *acc* cDNAs encoding ACC with those of the genes from *E. coli* (Toh *et al.*, 1993).

A modified Western blotting technique was used to identify biotinylated proteins in crude extracts of *S. coelicolor* grown on complex medium (Fig. 2). Essentially the same pattern of bands was seen in extracts obtained from cells grown on minimal medium (data not shown). Three major biotinylated proteins were visualized, with approximate molecular masses of 97.5, 88 and 70 kDa, respectively. This pattern was the same if the samples were boiled in SDS immediately after preparation of cell-free extracts, or were stored before SDS-PAGE (data not shown), suggesting that the smaller polypeptides were not derived by proteolytic degradation of the larger species during storage. No major biotinylated protein of less than 70 kDa was visualized when PAGE gels with a higher degree of cross-linking were run. The result contrasts with the situation in most other bacteria, for which the BCCP component of ACC is 22.5 kDa in size (Fall, 1979).

The pattern of biotinylated proteins in *S. coelicolor* is more complex than that seen in *E. coli* which contains a single biotinylated protein, the BCCP of ACC (Cronan & Rock, 1987). However, other Gram-negative species (e.g. *Pseudomonas aeruginosa*, Best & Knauf, 1993) contain more than one biotinylated protein which may reflect a greater metabolic diversity in soil organisms compared with enteric bacteria. A homologue of the BCCP from *Saccharopolyspora hirsuta* has been cloned (Legouill *et al.*,

Fig. 2. Western blot of SDS-PAGE of a cell-free extract of *S. coelicolor*. Lanes: 1 biotinylated protein standards with molecular masses indicated; 2, crude extract (0.215 mg protein). The blot was developed by incubation with streptavidin-peroxidase (see Methods).

1993) and the flanking DNA found to contain acyl carrier protein, ketosynthase and ketoreductase genes (the usual signature of a polyketide cluster). Hence it may be that the actinomycetes have a number of BCCPs, some of which may be associated with gene clusters for antibiotic biosynthesis.

All of the biotinylated proteins of *S. coelicolor* were larger than the biotinylated components of ACC found in other bacteria (Fall, 1979). Hence, the purification of one of these proteins based on its size and biotin content alone was unlikely to identify the required protein. Purification based on enzyme activity was necessary to identify the biotinylated protein in *S. coelicolor* which was associated with ACC activity.

ACC and PCC assays

Cell-free extracts of *S. coelicolor* grown on minimal and complex media were assayed for ACC activity using various concentrations of substrate and in the presence or absence of citrate or KCl, which are known to activate the mammalian and plant enzymes, respectively (Holland *et al.*, 1984; Nikolau *et al.*, 1981). Although ATP-dependent fixation of ^{14}C into acid-stable products was observed in some extracts, this activity was completely lost on fractionation of the extracts, and could not be regained by combining fractions.

The absence of both a detectable ACC activity and a biotinylated protein of the expected size for a bacterial BCCP led us to assay cell-free extracts of *S. coelicolor* for PCC activity. PCC catalyses a reaction analogous to that of ACC, with propionyl-CoA as substrate and methylmalonyl-CoA as product. In several species, one enzyme with dual substrate specificity catalyses the carboxylation of both acetyl- and propionyl-CoA. Whereas ACCs comprise three different types of subunits, these enzymes with dual substrate specificity have a subunit structure typical of PCCs rather than ACCs, containing equal

Table 1. Purification of PCC from *S. coelicolor* cells

ND, not determined (see Results and Discussion).

Step	PCC activity (mU ml ⁻¹)	Volume (ml)	Total PCC activity (mU)	Yield (%)	Protein concn (mg ml ⁻¹)	PCC specific activity (mU mg ⁻¹)
Crude extract	3.93	86	338	100	5	0.786
30–60% (NH ₄) ₂ SO ₄ pellet	2.75	60	165	49	ND	ND
DEAE-Sephacel eluate	1.6	52	83.2	25	ND	ND
Avidin-Sepharose eluate	2.87	17	49	14	ND	ND
Mono-Q fraction 24	4.41	0.5	2.2	3.4 (fractions 24–26)	ND	ND
fraction 25	10.23	0.5	5.11		0.05	204.6
fraction 26	8.31	0.5	4.15		ND	ND

Fig. 3. SDS-PAGE of protein fractions obtained during purification of PCC. Lanes: 1, molecular mass standards; 2, eluate with PCC activity from Avidin-Sepharose column; 3–8, eluates from MonoQ column, fractions 22–27, respectively. The gel was stained with silver and the PCC activity in each MonoQ fraction is shown by the bar-chart above each track. PCC activity was eluted from the column at 0.299 M NaCl (see Methods).

amounts of two distinct subunit types (Erfle, 1973; Hunaiti & Kolattukudy, 1982; Meyer & Meyer, 1978). The larger subunit contains biotin and bears the biotin carboxylase activity, while the smaller subunit lacks biotin and bears the carboxyl transferase activity (Toh *et al.*, 1993).

PCC activity of approximately $0.8 \text{ mU (mg protein)}^{-1}$, slightly higher than that seen in other bacteria, was detected in cell-free extracts of *S. coelicolor* grown on complex medium. Recently, it has been demonstrated (Kao *et al.*, 1994), that *S. coelicolor* will synthesize significant quantities of the cyclic macrolactone of erythromycin (which is known to be derived from methylmalonyl-CoA) when genes of the erythromycin biosynthetic cluster are introduced. This implies that, although *S. coelicolor* is not known to make a natural secondary metabolite derived from methylmalonyl-CoA, there must

Fig. 4. (a) N-terminal sequencing of the α subunit of PCC from *S. coelicolor* and comparison with similar proteins. The two sequences derived from the 88 kDa subunit of *S. coelicolor* PCC (see Results and Discussion) are shown as 4 and 5. Proteins with high similarity were: 1, propionyl-CoA carboxylase α subunit from rat liver (Browner *et al.*, 1989); 2, biotin carboxylase from *Mycobacterium tuberculosis* (Norman *et al.*, 1994); 3, biotin-containing protein from *Mycobacterium leprae* (Norman *et al.*, 1994). (b) Oligonucleotide designed against the N-terminal sequence of the α subunit of PCC from *S. coelicolor*. The choice of preferred codons was made with reference to Wright & Bibb (1992). (c) N-terminal sequence of the 66 kDa component of *S. coelicolor* PCC.

be a plentiful supply of that precursor as substrate for the heterologous gene products when they are expressed. PCC is the prime candidate for supply of methylmalonyl-CoA.

Purification of PCC

PCC was purified by a combination of $(\text{NH}_4)_2\text{SO}_4$ fractionation, anion-exchange chromatography and Avidin-Sepharose affinity chromatography (Table 1). The PCC protein was unstable during purification and the preparation typically gave a low yield of active enzyme. To facilitate speedy purification and to conserve material, protein estimation was only carried out at the beginning and the end of the procedure. SDS-PAGE showed that the purified sample contained three protein species (88, 70 and 66 kDa), the two largest species containing biotin as judged by Western blotting (data not shown). In the three most active fractions, the 88 kDa and 66 kDa species were present in a ratio of approximately 1:1 (Fig. 3). The 70 kDa species was not present in the same stoichiometry as the other species, nor was there any correlation between its presence in a fraction and the PCC activity of that fraction (Fig. 3). Since PCC is expected to be a heterodimer, it is likely that this protein is a contaminant, possibly involved in the transfer of CO_2 in another reaction, or generated by proteolysis of the 88 kDa protein.

N-terminal protein sequence of PCC subunits

The N-terminal sequences of the two subunits of PCC were determined (Fig. 4a, c). Comparison of the sequence of the 66 kDa subunit (Fig. 4c) with protein sequences in the NCBI database revealed no significant similarities. This is perhaps not surprising as, in other systems, these

Fig. 5. Restriction map of *pccA* locus in *S. coelicolor*. The predicted ORF of *pccA* is shown by the shaded arrow. Subclones which were used as templates for sequencing are shown in black, with arrows to indicate the direction of sequencing. Of the regions which were sequenced, the deduced peptide sequence identities were: 1, 65% to the BCCP from *M. tuberculosis* (Norman *et al.*, 1994); 2, 58% to the BCCP from *M. leprae* (Norman *et al.*, 1994); 3, 31% to the human PCC α subunit, including the biotin attachment site close to the C terminus.

carboxyl transferase subunits confer specificity for substrate to the enzyme complex and have poor sequence similarity (Li & Cronan, 1992b). Sequencing of the 88 kDa band from the gel yielded two sequences (Fig. 4a), indicating the presence of two protein species within the sample; however, these sequences were very similar and showed high similarity with α subunits of PCC (Browner *et al.*, 1989) and to other biotin carboxylases (Fig. 4a). It is unclear whether these sequences are isoforms of this subunit of PCC or whether the second peptide is a proteolytic fragment of the first, containing sequencing errors.

Oligonucleotide design and cloning of the *pccA* gene

The N-terminal protein sequence of the 88 kDa subunit of PCC from *S. coelicolor* was used to design an oligonucleotide probe with which to identify the *pccA* gene (Fig. 4b). Unique bands were obtained on hybridization of the oligonucleotide to genomic DNA from *S. coelicolor* which had been digested with a variety of restriction enzymes (data not shown). A 6.5 kb *Bam*HI/*Sph*I fragment of genomic DNA was cloned as described in Methods. DNA sequencing from the *Sph*I site and comparison of the deduced amino acid sequence obtained with those of known PCC sequences showed that the region cloned contained only the 5' end of the *pccA* gene. Attempts to clone the entire coding sequence on either a *Bam*HI or a *Sal*I fragment (12 kb and 3.5 kb, respectively) were unsuccessful due to rearrangement of the recombinant plasmids. However, it was possible to clone the entire *pccA* gene on a 2.7 kb *Pst*I fragment which

contained only a limited amount of additional *S. coelicolor* DNA upstream of *pccA* (approximately 150 bp; see Fig. 5). It is possible that the upstream sequences in the *Bam*HI and *Sal*I fragments contained a promoter which enabled transcription of the gene and that the gene product was not tolerated in *E. coli*, which does not itself possess a PCC.

The approximate location of the *pccA* coding sequence within the *Pst*I fragment was determined by partial sequencing of the insert (see Fig. 5). By comparison with other proteins in the database, it is likely that the *Pst*I fragment contained the entire *pccA* gene from *S. coelicolor*.

The results presented here are a first step in characterization of the pathway of malonyl-CoA biosynthesis in a streptomycete. The profile of biotinylated proteins in *S. coelicolor* showed that the ACC in this organism was (1) a very minor component in cell-free extracts, (2) unstable, or (3) atypical of both the known bacterial multienzyme complexes and the vertebrate multifunctional enzymes. The inability to detect ACC activity under a variety of conditions, although significant PCC activity was found, may point to a central role for the latter enzyme in cellular metabolism. One possible interpretation of the result might be that malonyl-CoA is derived from methyl-malonyl-CoA by the action of a demethylase (the reaction is entirely plausible), and this could explain the inability to detect ACC activity. On the other hand, PCC may also act *in vivo* as an ACC as is the case in other organisms (Erfle, 1973; Hunaiti & Kolattukudy, 1982; Meyer & Meyer, 1978). If so, it is surprising that we were unable to detect any ACC activity in the purified PCC – this could be due to loss of another component of the complex during purification or to our failure to activate ACC under the assay conditions used. A combination of biochemical and molecular genetic approaches will be required to address these possibilities.

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