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Primary Structure of the Monomer of the 12S Subunit of Transcarboxylase as Deduced from DNA and Characterization of the Product Expressed in *Escherichia coli*

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Transcarboxylase from *Propionibacterium shermanii* is a complex biotin-containing enzyme composed of 30 polypeptides of three different types: a hexameric central 12S subunit to which 6 outer 5S subunits are attached through 12 1.3S biotinyl subunits. The enzyme catalyzes a two-step reaction in which methylmalonyl coenzyme A and pyruvate serve as substrates to form propionyl coenzyme A (propionyl-CoA) and oxalacetate, the 12S subunit specifically catalyzing one of the two reactions. We report here the cloning, sequencing, and expression of the 12S subunit. The gene was identified by matching amino acid sequences derived from isolated authentic 12S peptides with the deduced sequence of an open reading frame present in a cloned *P. shermanii* genomic fragment known to contain the gene encoding the 1.3S biotinyl subunit. The cloned 12S gene encodes a protein of 604 amino acids and of M_r 65,545. The deduced sequence shows regions of extensive homology with the β subunit of mammalian propionyl-CoA carboxylase as well as regions of homology with acetyl-CoA carboxylase from several species. Two genomic fragments were subcloned into pUC19 in an orientation such that the 12S open reading frame could be expressed from the *lac* promoter of the vector. Crude extracts prepared from these cells contained an immunoreactive band on Western blots (immunoblots) which comigrated with authentic 12S. The *Escherichia coli*-expressed 12S was purified to apparent homogeneity by a three-step procedure and compared with authentic 12S from *P. shermanii*. Their quaternary structures were identical by electron microscopy, and the *E. coli* 12S preparation was fully active in the reactions catalyzed by this subunit. We conclude that we have cloned, sequenced, and expressed the 12S subunit which exists in a hexameric active form in *E. coli*.

Transcarboxylase (EC 2.1.3.1) is made up of three types of subunits, a central cylindrical hexameric subunit termed 12S to which are attached 6 dimeric subunits termed 5S via 12 biotinyl subunits termed 1.3S (24, 27). As for other biotin enzymes, the overall reaction catalyzed by transcarboxylase consists of two partial reactions. However, in contrast to other biotin enzymes, the reaction does not involve the fixation of CO_2 or a decarboxylation but rather a carboxyl transfer (23):

$$CH_3\underset{COO^-}{CH}COSCoA + \text{1.3S-biotin} \overset{12S}{\rightleftharpoons} CH_3CH_2COSCoA + \text{1.3S-biotin–}COO^- \quad (1)$$

$$CH_3COCOO^- + \text{1.3S-biotin–}COO^- \overset{5S}{\rightleftharpoons} {}^-OOCCH_2COCOO^- + \text{1.3S-biotin} \quad (2)$$

$$\text{Sum: MM-CoA + pyruvate} \overset{\text{transcarboxylase}}{\rightleftharpoons} \text{propionyl-CoA + oxalacetate} \quad (3)$$

The 12S subunit specifically catalyzes the transfer of the carboxyl group of methylmalonyl coenzyme A (MM-CoA) to the biotin of the 1.3S subunit, whereas the 5S subunit specifically catalyzes the transfer of the carboxyl group from biotin to pyruvate (2).

The 12S subunit is composed of six identical monomers ($M_r \cong 60{,}000$). There are 12 CoA ester binding sites on the 12S subunit, two per monomer (17). The complete 26S form of transcarboxylase has six outer 5S subunits but dissociates to an 18S form of the enzyme at neutral pH, with only three 5S subunits attached to one of the faces of the 12S subunit (28). These findings have been interpreted to indicate that the two faces of the cylindrical subunit are distinct and that monomers associate in a parallel orientation since an antiparallel arrangement would produce identical faces and preclude such unilateral dissociation. The fact that there are two CoA ester sites per monomer of the 12S subunit and there are two binding sites for the 1.3S subunits per monomer, six on each face, has led to the speculation that 12S monomers may have arisen by gene duplication and fusion (29).

The 12S subunit has been crystallized and is now being investigated by X-ray crystallography (20, 26). As a source of material for structural studies and for investigation of the functional domains of the 12S protein, cloning and expression studies were carried out. We screened a *Propionibacterium shermanii* genomic library with the 1.3S gene (14) as a probe to isolate a genomic fragment which contained the other subunit genes. We report here the sequencing of the 12S gene, expression of the 12S subunit in *Escherichia coli*, and demonstration of its functional activity.

MATERIALS AND METHODS

Cloning and sequencing the 12S subunit gene. A 7.1-kb fragment was isolated from a *P. shermanii* genomic library (pPS-Lib), using a 1.7-kb 1.3S-containing clone (14) as a

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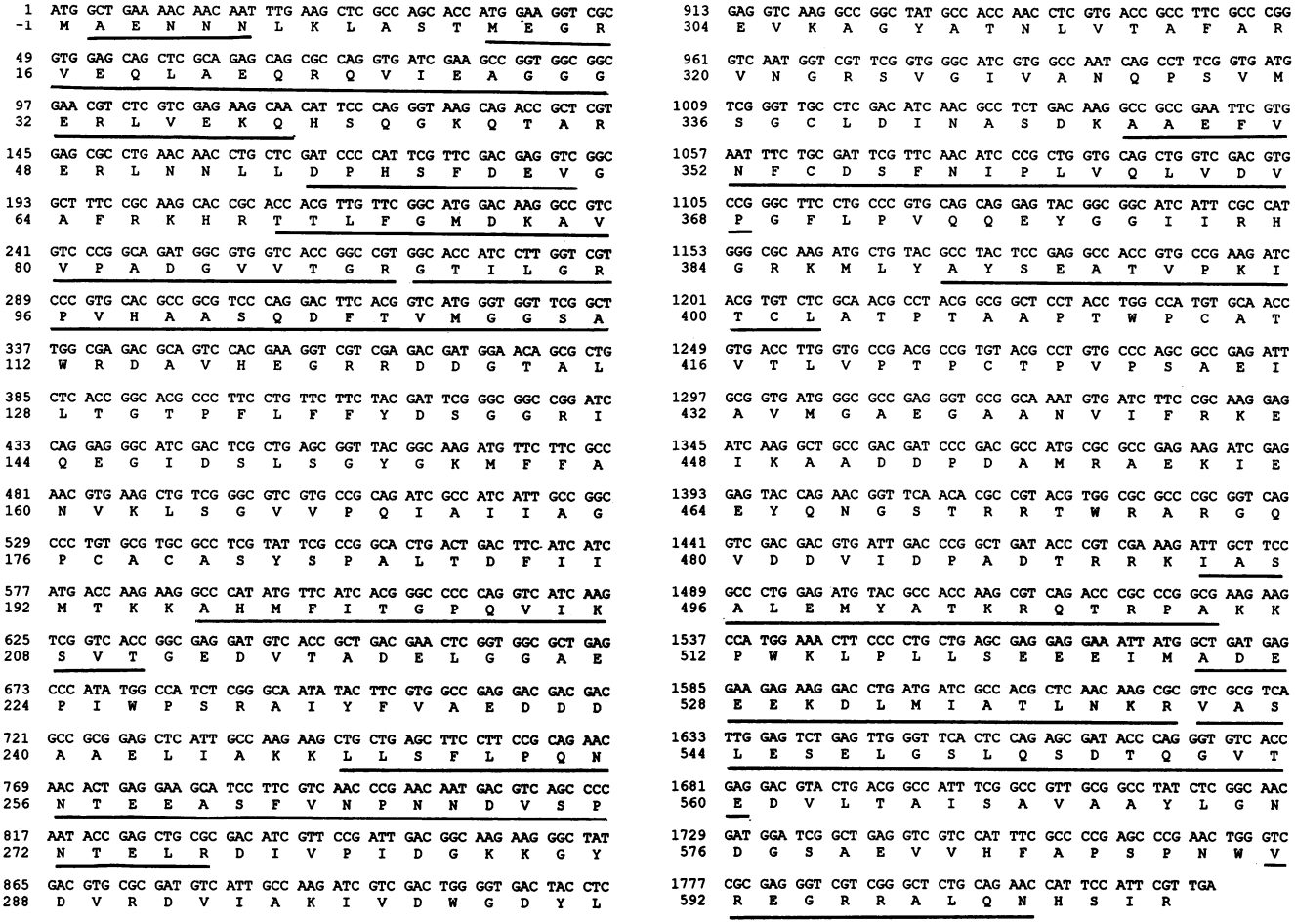

FIG. 1. DNA sequence of the gene encoding the monomer of 12S subunit of transcarboxylase and its deduced primary amino acid sequence. The DNA sequence of the 12S open reading frame and its deduced amino acid sequence are shown. The numbering (1 to 604) is that of the the mature authentic protein, which does not have an N-terminal methionine. The underlined sequences are those of peptides which were matched by the amino acid sequencing.

probe. The library contained size-fractionated 5- to 10-kb fragments generated by partial digestion with *Sau*3A and ligated into the *Bam*HI site of pUC9. The 7.1-kb fragment was extensively restriction mapped, and random fragments were subcloned into M13mp9 for sequencing by the dideoxy-chain termination method (5), using the large fragment (Klenow) of DNA polymerase I or Sequenase. The sequence from overlapping restriction fragments was used to generate the entire sequence of the region. The identity of the open reading frame encoding the monomer of the 12S subunit was made by matching amino acid sequences derived from peptides isolated from trypsin-treated transcarboxylase and cleavage with CNBr of 12S with the deduced sequence from the 7.1-kb clone. Regions in which discrepancies existed with the protein data were resequenced from several independent subclones. The DNA sequence and the deduced amino acid sequence of the 12S monomer open reading frame are shown in Fig. 1.

Expression of the 12S subunit gene in *E. coli*. The 12S subunit was initially expressed from a 3.2-kb *Sph*I-to-*Bgl*II fragment subcloned into pUC19. A partial restriction map and the vector are diagrammed in Fig. 2. This fragment encoded the COOH-terminal portion of the 5S subunit gene, the entire 12S gene, and the 1.3S gene. The subclone, termed plac12S+1.3S, was positioned such that the *lac* promoter was able to direct the synthesis of the 12S and 1.3S subunits. The subclone was used to transform *E. coli* JM109 and CSH26, and the expressed 12S and 1.3S subunits were detected by Western blotting (immunoblotting) as described previously (1), using an antitranscarboxylase antiserum. Subsequently, plac12 S+1.3 S was digested with *Pst*I, which cleaves at amino acid 599 in the 12S gene and removes the 1.3S gene. The COOH terminus of the 12S subunit gene was reconstructed by cloning complementary synthetic oligonucleotides which encoded amino acids 599 to 604 and generated *Pst*I ends when annealed. The resulting subclone was termed plac12S and was used to express the 12S subunit as described below.

Purification of wild-type 12S subunit. Plasmid plac12S in *E. coli* JM109 was grown in MinA (19) medium to early stationary phase, harvested by centrifugation, and washed in 25 mM potassium phosphate buffer (pH 7.2) containing 1 mM dithiothreitol (DTT) and 0.1 mM each EDTA, phenylmethylsulfonyl fluoride (PMSF), and sodium azide (buffer A). Washed cells were frozen at −70°C.

For purification, 30 g of cells was suspended in 90 ml of buffer A and lysed by two passages through a French pressure cell. The lysate was cleared by centrifugation at

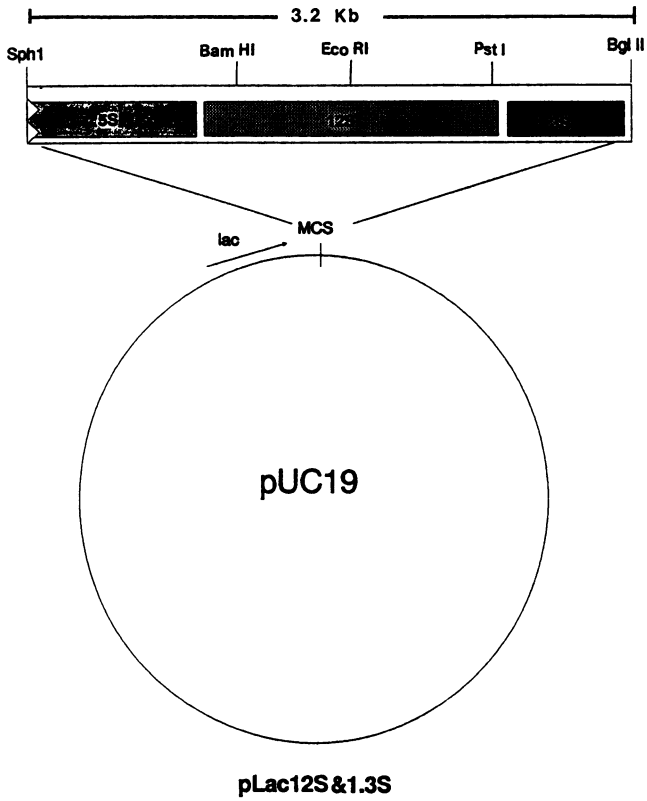

FIG. 2. Construct for expression of the gene encoding the monomer of the 12S subunit. plac 12S+1.3S contains the 3.2-kb *Sph*I-to-*Bgl*II fragment cloned downstream of the *lac* promoter of pUC19. The *Pst*I site at position 599 in the 12S sequence is indicated and was used to generate plac 12 S, an expression construct lacking the gene encoding the 1.3S subunit. MCS, multiple cloning site.

TABLE 1. Purification of the wild-type 12S subunit of transcarboxylase expressed in *E. coli*

Sample	Total protein (mg)	Total activity (μmol/min)	Sp act[a]	Fold purification	% Recovery
Crude extract	1,680	481	0.3		100
0–45% $(NH_4)_2SO_4$	1,151	453	0.4	1.3	94
DE-52	245	412	1.7	5.7	86
Cellulose phosphate	74	367	5.0	16.7	76
Bio-Gel A-1.5m	23	302	13.1	43.7	63

[a] Measured by assaying partial reaction 1 as described in Materials and Methods and expressed as micromoles of 1.3S carboxylated per minute per milligram of 12S subunit.

27,000 × *g* for 60 min and dialyzed against buffer A (all steps in the purification were performed at 4°C). The dialysate was loaded on a Whatman DE-52 column (400-ml bed volume) previously equilibrated with buffer A and eluted with increasing concentrations of potassium phosphate buffer in three steps of 100, 200, and 500 mM. The major portion of the 12S subunit was eluted by 500 mM potassium phosphate buffer. The fractions containing 12S from the 500 mM eluate were identified by Western blotting, pooled, and dialyzed against 25 mM potassium phosphate buffer (pH 6.5), containing 1 mM DTT and 0.1 mM each EDTA, PMSF, and sodium azide (buffer B). The dialysate was passed over a cellulose phosphate column (Whatman P11; 200-ml bed volume) which had previously been equilibrated with buffer B, and 12S was eluted with increasing concentrations of potassium phosphate buffer as described above for the DE-52 column. The 12S protein-containing fractions from the 500 mM eluate were pooled and dialyzed against 250 mM potassium phosphate buffer (pH 6.5) containing 1 mM DTT and 0.1 mM each EDTA, PMSF, and sodium azide (buffer C). The dialyzed sample was size fractionated on Bio-Gel A-1.5m, using buffer C. The final preparation was homogeneous, as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Typical recovery was 8 mg of 12S from 30 g of cells. Table 1 summarizes the procedure.

Determination of activity in the overall reaction. Transcarboxylase was assayed in the forward direction by monitoring the formation of oxalacetate via a coupled assay system using malate dehydrogenase and, spectrophotometrically, measuring the decrease in NADH absorbance as previously described (25).

Measurement of partial reaction 1 with the 12S subunit. The partial reaction was conducted in a water bath at 25°C in 7 ml of borosilicate glass vials (Fisher Scientific). The vials are placed in a tilted test tube rack so that the solution accumulates at one edge, and the solutions are introduced at this edge by using Hamilton syringes. The additions are made in the following order: 30 μl of 1.0 M phosphate buffer (pH 6.6) and H_2O so that the final volume will be 85 μl, and then the selected amount of an aqueous solution of the 1.3S subunit and 10 μl containing 0.6 μg of the 12S subunit in 0.5 M phosphate buffer (pH 6.6). The reaction is initiated by the addition of 25 μl of 0.0625 mM phosphate buffer (pH 6.6) containing 1.4 nmol of [3-^{14}C]MM-CoA and 0.03 U of MM-CoA racemase. Only one isomer (*S*) of the MM-CoA is enzymatically active, and the racemase keeps the two forms at equilibrium as the active form is utilized. The vial is immediately shaken by hand; after 60 s, 50 μl of 1 M HCl is injected into the vial to stop the reaction, and the vial is again shaken prior to drying at 45°C under vacuum. The dried samples are then dissolved in 200 μl of H_2O; after 5 min (with occasional shaking), 2.8 ml of scintillant (Formula-963; NEN Research Products) is added, and the radioactivity is determined from the average of duplicate counts at 10 min. In each experiment, duplicate controls that contain only the 12S subunit are included. The difference in acid-stable counts of the control from that observed in the presence of the 1.3S subunit is a measure of the amount of carboxyl transfer that has occurred. This carboxyl transfer activity is determined as described in Table 2, footnote *a*.

Binding assay with [3-^{14}C]MM-CoA. To determine the number of MM-CoA binding sites on the expressed 12S, 0.1 nmol of 12S was mixed with various amounts of [3-^{14}C]MM-CoA in 250 μl of 0.2 M phosphate buffer (pH 6.5) containing 0.1 mM each EDTA and DTT and incubated at room temperature for 10 min. This mixture was then subjected to ultrafiltration with PM-10 membranes, and the protein-bound radioactivity was analyzed to determine bound substrate as described by Poto et al. (17).

Isolation of peptide labeled with [^{14}C]*p*-azidobenzoyl-CoA. Transcarboxylase was treated with the photoaffinity label [^{14}C]*p*-azidobenzoyl-CoA as described by Poto et al. (17). The 12S subunit was isolated from the labeled enzyme and digested with trypsin. By successive gel filtration (Sephadex G-50) and reverse-phase high-pressure liquid chromatography (HPLC) (C-18) chromatography, one major radioactive peak was isolated. From 66 nmol of transcarboxylase, about 40 nmol of peptide was obtained. The peptide was sequenced

in Earl Davies' laboratory by Brad McMillen, with the following results (the amino acids in parentheses were minor components): Asp (Glu, Ala), Pro, His, Ser, Phe, Asp (Val), Glu (Phe), Val. The sequence corresponds to amino acids 55 to 62 from the deduced DNA sequence.

HPLC analysis. The tryptic and CNBr digests were separated on a reverse-phase C-4 or C-8 column (Synchropak), using a Du Pont HPLC system. Peptides were resolved with a linear gradient formed from 0.1% trifluoracetic acid (TFA)–H_2O and 0.1% TFA–CH_3CN. Peptide elution was monitored at 220 nm. Selected peptides were subsequently repurified on a reverse-phase C-18 column prior to sequence analysis.

NH_2-terminal and amino acid analysis. The amino-terminal residue of the isolated peptides was determined by the dansyl method (29). For amino acid composition analysis, the peptides were hydrolyzed in vacuo in 6 N HCl for 18 h, and the proteins were hydrolyzed for 24, 48, and 72 h at 110°C. The amino acid composition was determined by a modified PTC method as described by Phillips and Wood (16).

The COOH terminus. BNPS-Skatole [2-(2'-nitrophenylsulfenyl)-3-bromoindolenine] was used to selectively cleave tryptophanyl peptide bonds (3) and to isolate a peptide from the COOH terminus of the 12S subunit. To a solution of 12S subunit (2 mg in 1.2 mg of 50% acetic acid), 25 mg of BNPS-Skatole (Pierce Chemical Co.) in 0.6 ml of glacial acetic acid (final volume, 1.8 ml) was added. The reaction was allowed to proceed at room temperature in the dark with stirring for 20 h. Then 12 mg of thioglycolic acid in water was added to reduce the oxidation of the methionines, and the mixture was incubated at room temperature for another 4 h. The lyophilized sample was dissolved in 0.1% TFA and analyzed on a C-8 reverse-phase column. The peptides were separated by using a gradient of 0.1% TFA–H_2O and 1% TFA–CH_3CN and subjected to N-terminal sequencing. The derived sequence was Val, Arg, Glu, Gly, X, X, Ala, Leu, Gln, Asn, consistent with amino acids 591 to 600 of the deduced sequence of the 12S subunit shown in Fig. 1.

Sequence determination. An Applied Biosystems model 470A sequencer was used for sequence analysis of the purified peptides (8). Phenylthiohydantoins were identified by complementary HPLC systems (4). Quantitative evaluation of the phenylthiohydantoin-derived amino acids was done as described by Smithies et al. (21) and Machleidt and Hofner (13).

Circular dichroism measurement. Circular dichroism spectra (190 to 250 nm) were recorded at 25°C with a JASCO 600 spectropolarimeter, using 0.1-cm cells. The protein concentration was 100 μg/ml. Mean residue ellipticity was calculated by using a mean residue weight of 110. For the calculations of the secondary structure components, the following proteins were used as a data base: myoglobin, lysozyme, RNase A, papain, cytochrome *c*, hemoglobin, and chymotrypsin A. Secondary structure prediction analysis of the 12S sequence was performed by using the University of Wisconsin Genetics Computer Group sequence analysis software package (version 6.2).

Electron microscopy. Electron microscopy was performed with a JEM-100C (JEOL) instrument. The protein samples were processed for microscopy as described previously (6, 28).

Nucleotide sequence accession number. The GenBank accession number for the sequence shown in Fig. 1 is LO4196.

RESULTS AND DISCUSSION

Primary structure of the 12S subunit. A 7.1-kb *P. shermanii* genomic fragment containing the 1.3S gene was isolated and partially sequenced. Two open reading frames upstream of the 1.3S gene were identified. The DNA and deduced amino acid sequences of one of these open reading frames are shown in Fig. 1. The DNA sequence contained an open reading frame of 1,812 nucleotides, encoding a protein of 604 amino acids with a calculated molecular weight of 65,545.

The N terminus of the 12S subunit was determined by Zwolinski et al. (29) to begin with an Ala residue. This was confirmed when the first five N-terminal amino acid residues of the 12S subunit from *P. shermanii* were identified by amino acid sequence analysis and shown to match residues 1 to 5 of the deduced sequence from the open reading frame shown in Fig. 1 (amino acid −1 is the presumed initiating methionine). The 12S subunit C-terminal peptide was isolated following BNPS-Skatole cleavage of the cloned *E. coli* 12S subunit, and the 10-residue partial protein sequence obtained corresponds to residues 591 to 600. A TGA stop codon exists in this reading frame at position 605, implying that this is the COOH terminus of the encoded protein. A total of 224 amino acid residues of the 12S subunit were determined by protein sequence analysis of 12 purified peptides underlined in Fig. 1; except for residue 560, total agreement with the deduced DNA sequence was found. In general, the amino acid compositions of the authentic 12S subunit and *E. coli*-expressed 12S subunit are in good but not perfect agreement with the composition deduced from the DNA sequence (data not shown).

From the compositional similarities and sequence identity between the deduced sequence and the amino acid sequence derived from authentic 12S peptides, we conclude that we have cloned and sequenced the gene encoding the monomer of the 12S subunit.

Expression of the gene for the monomer of the 12S subunit in *E. coli*. We have previously developed in *E. coli* expression system for the 1.3S subunit of transcarboxylase (14) and attempted to establish a similar system for the 12S subunit. Two fragments, a 2.4-kb *Sph*I-to-*Pst*I fragment and a 3.2-kb *Sph*I-to-*Bgl*II fragment derived from the original 7.1-kb genomic clone, were subcloned into pUC19 such that the 12S gene was downstream of the *lac* promoter in the vector (Fig. 2). The longer fragment contains DNA encoding the COOH terminus of the 5S subunit and the entire 12S and 1.3S subunits. The shorter fragment does not encode the 1.3S gene. In Fig. 3, a Western blot of total cell extracts of *E. coli* containing these fragments and a control extract derived from the *E. coli* host strain (JM109) and the pUC19 vector is shown. The extracts were fractionated by SDS-PAGE, transferred to polyvinylidene difluoride membranes, and incubated with an antiserum against transcarboxylase (1) and an alkaline phosphate-linked second antibody. From a comparison of the immunoreactive products with authentic subunits isolated from transcarboxylase, it is evident that the subcloned fragments encode a protein which comigrates with the monomer of the authentic 12S subunit from *P. shermanii* (Fig. 3, lanes 1 and 2) and that the longer fragment also encodes the 1.3S subunit.

Purification and enzymatic activity of *E. coli*-expressed 12S. The 12S subunit expressed from the 3.2-kb *Sph*I-to-*Bgl*II fragment in pUC19, using *E. coli* JM109 as a host, was purified to apparent homogeneity by a three-step procedure as described in Materials and Methods (Table 1). The purified recombinant protein showed a single band by SDS-

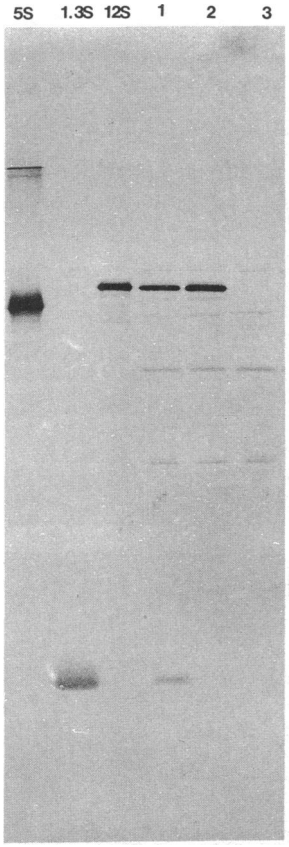

FIG. 3. Western blot analysis of the 12S subunit expressed in *E. coli*. Samples were crude extracts of *E. coli* transformed with a plasmid encoding 12S plus 1.3S (lane 1), or 12S (lane 2) or with a pUC19 vector control (lane 3). After separation by SDS-PAGE as described in Materials and Methods, samples were electroblotted onto polyvinylidene difluoride membranes and incubated with an anti-transcarboxylase antiserum followed by alkaline phosphatase goat anti-rabbit immunoglobulin G. Authentic 5S, 12S, and 1.3S subunits isolated from *P. shermanii* transcarboxylase served as standards.

PAGE at ~60 kDa and was indistinguishable from authentic 12S by negative-stain electron microscopy. To determine whether the expressed and isolated 12S was in its native hexameric configuration, samples of the *E. coli*-expressed material were compared with authentic 12S by negative-stain electron microscopy. As shown in Fig. 4, circular or polygonal profiles (100 Å [1 Å = 0.1 nm] in diameter) with a stain-filled central hole are abundantly evident with the *E. coli*-expressed subunit and are similar to those observed with the authentic 12S subunit from *P. shermanii*. This feature represents a top view of the cylindrical structure of the 12S subunit. Rectangular or lozenge-shape profiles (70 by 100 Å) with stain penetrating along both axes are likewise seen. This is an edgewise view of the cylindrical structure. We conclude that 12S monomers expressed in *E. coli* have assembled to form the hexameric 12S subunit and appear in the electron microscope to be similar to authentic 12S.

As an additional method to compare the structure of the recombinant and authentic forms of the 12S subunit, circular dichroism spectra of each protein were determined (Fig. 5). The spectra were indistinguishable and indicate that the 12S subunit is composed of 38% α helix, 16% β sheet, 16% β turn, and 25% random coil.

The purified *E. coli*-expressed 12S subunit was tested for its ability to catalyze partial reaction 1 and to assemble with 1.3S and 5S subunits to form transcarboxylase and catalyze the overall reaction. The *E. coli*-expressed 12S was as active in catalyzing partial reaction 1 as the 12S subunit from *P. shermanii* (Table 2). Likewise, catalysis of the overall reaction (reaction 3) in combination with the 5S and 1.3S subunits was comparable to that with authentic 12S (Table 2). By these criteria, the 12S subunit expressed and isolated from *E. coli* was indistinguishable from the authentic 12S subunit isolated from *P. shermanii* transcarboxylase.

Binding assays with [3-^{14}C]MM-CoA demonstrated the ability of the 12S subunit isolated from *E. coli* to bind 12 CoA ester molecules per 12S hexamer. These experiments used Paulus ultrafiltration as described in Materials and Methods. The results were comparable to those of Poto et al. (17), who used intact transcarboxylase. Poto et al. could not accurately measure the CoA ester binding sites in isolated 12S subunits because of partial denaturation during preparation. Our studies with the *E. coli*-expressed 12S subunit avoid this problem, allowing binding site measurements to be performed accurately with the authentic substrate. Two CoA ester sites were found per 12S monomer. Kinetic studies with the cloned 12S subunit allowed calculation of the apparent K_m value of 4.4 μM for MM-CoA. This determination is in agreement with the reported value for the enzyme from *P. shermanii* (15).

A single peptide containing residues 55 to 62 was identified with the cross-linkable photoaffinity label *p*-azidobenzoyl-CoA. However, a second cross-linked peptide corresponding to the hypothesized second CoA ester site on each 12S monomer was not identified. Possibly, the *p*-azidobenzoyl-CoA peptide containing residues 55 to 62 and the sequence from 77 to 115 (see below) shown in Fig. 1 are both parts of the same CoA ester site. Likewise, sequence 306 to 343 might be the second CoA ester site as described below.

There are two CoA ester sites per monomer of the 12S subunit (17), and it has been suggested that the two sites may have arisen by gene duplication and fusion (29). While the data from the sequence of the 12S subunit do not provide significant evidence for such fusion, limited homology between positions 77 to 115 and 306 to 343 (Fig. 6A) might be the result of such duplication.

Sequence comparison. We have previously (18) noted remarkable stretches of sequence identity when comparing the β subunit of mammalian propionyl-CoA carboxylase (9, 10) with the 12S subunit of transcarboxylase. Significant homology between these two proteins was expected since the β subunit of propionyl-CoA carboxylase catalyzes the reverse of the 12S partial reaction (reaction 1). Transcarboxylase is also capable of catalyzing the carboxylation of acetyl-CoA (24), which involves a partial reaction identical to that of acetyl-CoA carboxylase (11, 22). Takai et al. (22) and Lopez-Casillas et al. (12) have shown that there is significant homology between portions of the sequences of mammalian acetyl-CoA carboxylase and mammalian propionyl-CoA carboxylase. Similar regions of sequence identity have recently been reported between the carboxyl transferase subunit of *E. coli* acetyl-CoA carboxylase and the 12S subunit of transcarboxylase (11). Figure 6 shows sequence comparisons between 12S and other carboxylases from mammalian and prokaryotic sources. There is strong homology between the sequences of 12S, *E. coli* acetyl-CoA carboxylase, and rat propionyl-CoA carboxylase in the region from 66 to 171 of the 12S subunit (Fig. 6B). There are also similarities between 12S and rat and human propionyl-

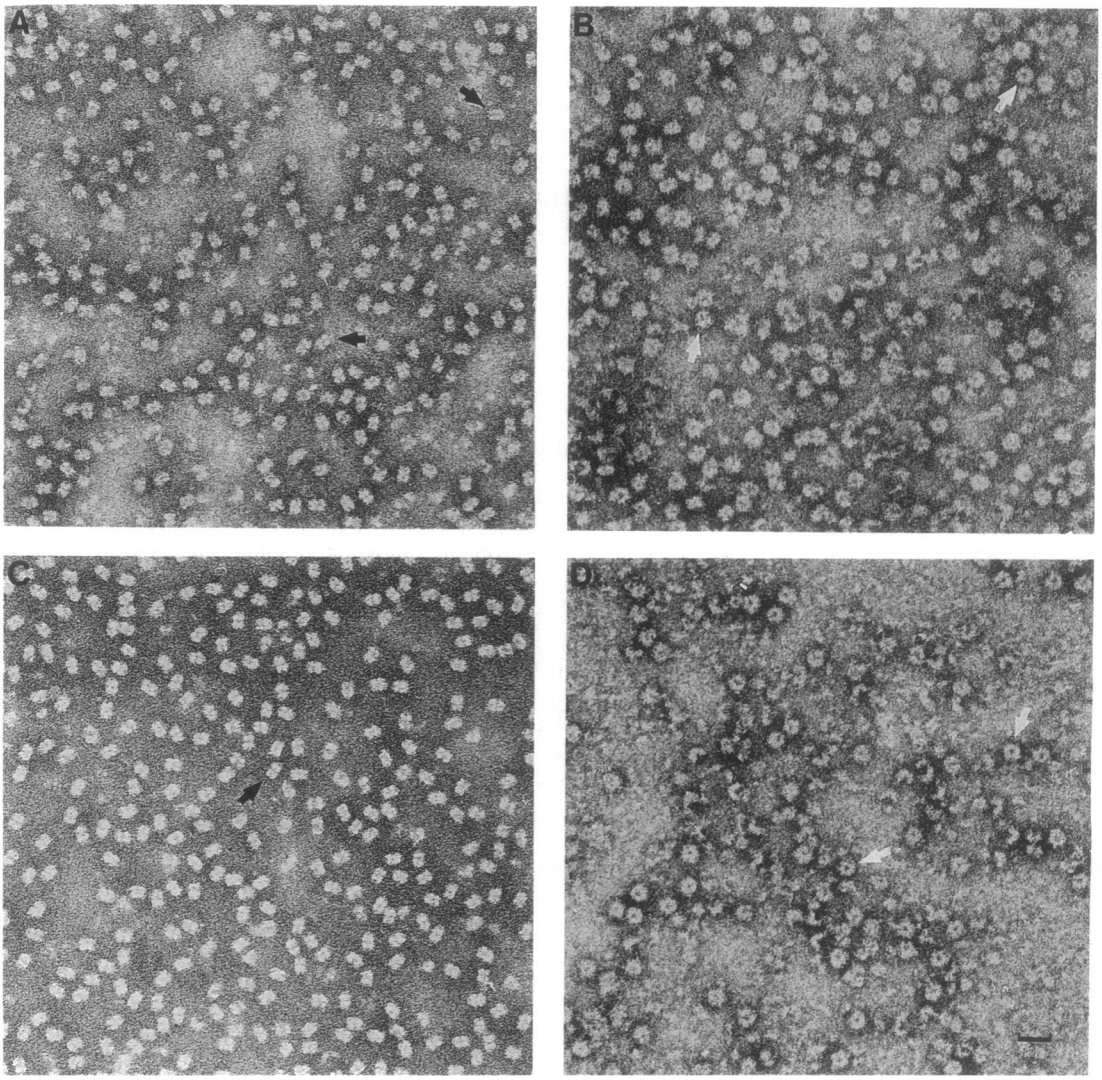

FIG. 4. Representative electron micrographs of *E. coli* 12S and *P. shermanii* 12S subunits of transcarboxylase. Profiles of 12S from *P. shermanii* (A, edge view; B, top view) and recombinant 12S (C, edge view; D, top view) are shown. Magnification, ×250,000; bar, 400 Å.

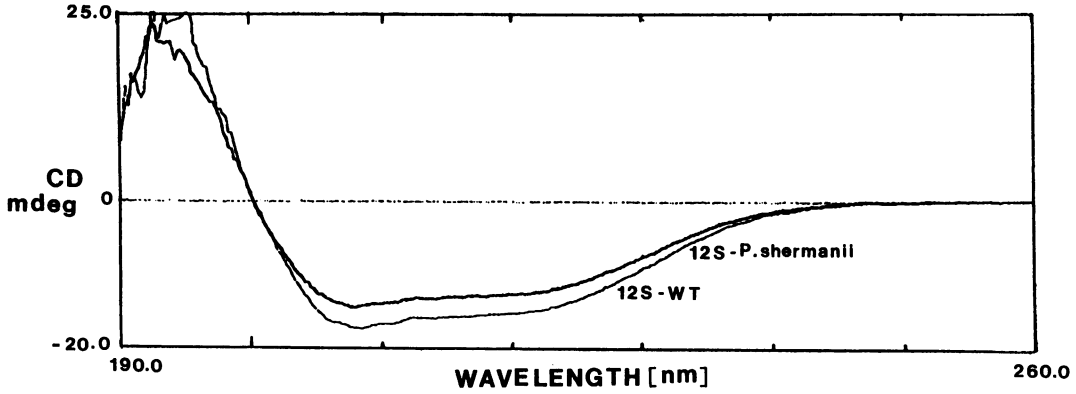

FIG. 5. Circular dichroism (CD) spectra of recombinant 12S from *E. coli* (12S-WT) and authentic 12S subunit from *P. shermanii*. The spectra were determined at 25°C as described in Materials and Methods. CD ellipticity in millidegrees (mdeg).

TABLE 2. Activity of *E. coli*-expressed 12S and 12S from *P. shermanii* in partial reaction 1 and overall reaction 3

12S	Sp act	
	Partial reaction 1[a] (μmol/min/mg of 12S subunit)	Overall reaction 3[b] (μmol of oxalacetate formed/min/mg of 12S subunit)
From *P. shermanii*	7.37	101.5
Wild type	13.4	135.4

[a] Assay mixtures (85 μl) contained 25 ng of wild-type 12S subunit, 8 nmol of 1.3S subunit, 4.36 nmol of [3-^{14}C]MM-CoA (7,620 cpm/nmol), and 35 μmol of phosphate buffer (pH 6.6). Temperature, 25°C; time of reaction, 30 s.
[b] Measured as described in Materials and Methods.

CoA carboxylases in the region from 260 to 415 (Fig. 6C). The homology between the 12S subunit and the β subunit of propionyl-CoA carboxylase extends over a much larger region (18) than it does with acetyl-CoA carboxylase. In accord with this observation, the quaternary structure of the β subunit of propionyl-CoA carboxylase closely resembles that of the 12S subunit (7). Perhaps the more confined homology with acetyl-CoA carboxylase is limited to the CoA ester site.

The results of this study show quite conclusively that the 12S subunit has been cloned. The protein product of the subunit gene when expressed in *E. coli* is assembled to the hexameric form and is fully active in partial reaction 1 and in the overall reaction. The availability of the clone for the 12S subunit now opens the opportunity for exploration of the CoA ester sites by selected mutations of the 12S subunit

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A
12S   (77) K A V V P A D G V V T G R G T I L G R P V H A A S Q D F T V M G G S A W R D A

12S   (306)K A G Y  A T N L V T A F A R V N G R S V G I V A N Q P S V M S G C L D I N A

B
12S            (66) R K H R T T L F G M D K A V V P A D G V V T G R
rPCCβ          (88) V E H R C A D F G MAAEK N K F P G D S V V T G R
EcACCβ         (89) K K Y K D R L A S A Q K E T G E K D ALV V  M K

12S     G T I L G R P V H A A S Q D F T V M G G S A W R D A V H E G
rPCCβ   G R I N G R L V Y V F S Q D F T V F G G S L S G A H A Q K I
EcACCβ  G T L Y G M P V V A A A F E F A F M G G S  M G S V V    G

12S     R R D D G T A L L T G T P F L F F Y D S G G R I Q E G I D S
rPCCβ   C K I M D Q A I T V G A P V I G L N D S G GAR I Q E G V E S
EcACCβ  A R F VRAVEQA L E D N C P L I C F S A S G GAR M Q E A L M S

12S     L S G Y G K M F F A N V K L S G V V P Q I A I I A G P C(177)
rPCCβ   L A G Y A D I F L R N V T A S G V I P Q I S L I M G P C(203)
EcACCβ  L M Q M A K T S A A L A K M Q ERG L P Y I S V L T D P  (201)

C
12S                                  (260)  A S F V N P N N D V
rPCCβ                                (288)  A S I R E C H   D P
hPCCβ                                (1)    R E C H D P S D R L

12S     S P N T E L R D I V P I D G K K G Y D V R D V I A K I V D W
rPCCβ   SDRLV P E L D T V V P L E S S K A Y N M L D I I H A  VID E
hPCCβ   V P    E L D T I V P L E S T K A Y N M V D I I H S V V D E

12S     G D Y L E V K A G Y A T N L V T A F A R V N G R S V G I V A
rPCCβ   R E F F E I M P N Y A K N I V I G F A R M N G R T V G I V G
hPCCβ   R E F F E I M P N Y A K N I I V G F A R M N G R T V G I V G

12S     N Q P S V M S G C L D I N A S D K A A E F V N F C D S F N I
rPCCβ   N Q P N V A S G C L D I N S S V K G A R F V R F C D A F S I
hPCCβ   N Q P K V A S G C L D I N S S V K G A R F V R F C D A F N I

12S     P L V Q L V D V P G F L P V Q Q E Y G G I I R H G R K M L Y
rPCCβ   P L I T F V D V P G F L PGT A Q E Y G G I I R H G A K L L Y
hPCCβ   P L I T F V D V P G F L PGT A Q E Y G G I I R H G A K L L Y

12S     A Y S E A T V P K I T C L A T P T A A P T W P C A T (415)
rPCCβ   A F A E A T V P K I T V I T R K A Y G G A Y D V M S (445)
hPCCβ   A F A E A T V P K V T V I T R K A Y G G A Y D V M S (155)
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FIG. 6. Comparison of the sequence of the monomer of the 12S subunit of transcarboxylase with those of the β subunit of propionyl-CoA carboxylase of rat and acetyl-CoA carboxylase of *E. coli*. Numbering is that of the 12S subunit (604 residues). The comparison is shown in two segments which represent the regions of maximum identity between the three sequences shown. Numbering in parentheses corresponds to the standard residue numbering of each polypeptide. Residues in boldface type are identical at the given position in all three proteins. Identity restricted to two of the sequences and positions of high similarity are not specifically noted. (A) Comparison of two regions within the 12S subunit, 77 to 115 (top line) and 306 to 343 (bottom line). (B) Sequence comparison of 12S with the β subunit of rat propionyl-CoA carboxylase (rPCCβ) and the β subunit of *E. coli* acetyl-CoA carboxylase (EcACCβ). The β subunit of propionyl-CoA carboxylase of rat consists of 541 residues, and the β subunit of acetyl-CoA carboxylase of *E. coli* consists of 304 residues. (C) Sequence comparison of 12S with the β subunits of rat and human propionyl-CoA carboxylase (rPCCβ and hPCCβ). The human β subunit of propionyl-CoA carboxylase consists of 156 residues.

gene. At the present time, no information is available concerning amino acid residues of the 12S subunit involved in interactions with the 1.3S biotinyl subunit necessary for activity in the partial reaction or residues involved in monomer interactions to form the stable 12S hexamer. Mutagenic studies designed to identify the CoA binding sites of the 12S subunit are presently underway.

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