

Cloning and nucleotide sequence of the *fabD* gene encoding malonyl coenzyme A-acyl carrier protein transacylase of *Escherichia coli*

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We report the cloning and nucleotide sequence of the gene encoding malonyl coenzyme A-acyl carrier protein transacylase of *Escherichia coli*. Malonyl transacylase has been overexpressed 155-fold compared to a wild-type strain. Overexpression of this enzyme alters the fatty acid composition of a wild-type *E. coli* strain; increased amounts of *cis*-vacenate are incorporated into the membrane phospholipids.

Malonyl transacylase; Malonyl-CoA; Acyl transferase; Fatty acid biosynthesis

1. INTRODUCTION

Malonyl coenzyme A-acyl carrier protein transacylase (malonyl transacylase) catalyzes a key reaction of fatty acid synthesis in bacteria and plants, the conversion of malonyl-CoA to malonyl-acyl carrier protein (ACP). Malonyl-ACP then acts as the two carbon donor in the elongation steps of fatty acid synthesis. A similar reaction occurs in fatty acid synthesis in fungi and mammals except that both the malonyl transacylase and ACP moieties are domains of the polyfunctional fatty acid synthases of these organisms. *E. coli* malonyl transacylase has been purified [1,2], and aspects of the enzymatic mechanism studied [3]. Mutants defective in the enzyme have also been isolated [4]. In this paper, we report the cloning, DNA sequence, and overexpression of malonyl transacylase. We also report the effects of overexpression on the fatty acid composition of the membrane phospholipids of *E. coli*.

2. MATERIALS AND METHODS

The bacterial strains used were all derivatives of *E. coli* K-12. Strain L48 (also called LA2-89) [4], carries a temperature-sensitive lesion in the *fabD* gene resulting in a malonyl transacylase of abnormal thermostability. *recA* derivatives of this strain were made either by P1 transduction or bacterial conjugation using a *Tn10* element closely linked to *recA1*. Standard recombinant DNA methods were used as were standard plasmids and host strains. DNA sequencing was done using the Sequenase kits from United States Biochemicals with both single-stranded and double-stranded templates. The sequence reported was obtained by complete sequencing of both DNA strands and was independently obtained in both laboratories. Protein expres-

sion studies were done using either the T7 polymerase system (for the truncated protein) [5], or the *tac* promoter of plasmid pCKR101 [6].

3. RESULTS

3.1. Sequence of *fabD*

The *fabD* gene was isolated from two different sources; lambda miniset phage 235 of the Kohara library [7] and from a minibank of *E. coli* DNA fragments in plasmid pACYC184 [8]. In both cases, the gene was isolated by complementation (or recombinational repair) of the *fabD* strain LA2-89 which encodes a temperature-sensitive enzyme. The gene carried by λ 235 was further localized by subcloning into high copy number plasmids. The minimal clone conferring growth at 42°C was sequenced and found to encode a truncated version of FabD lacking the last 21 amino acids. N-terminal sequencing of the truncated protein gave the sequence TQFAFVFPQG, and thus the initiation methionine is the most upstream of the three in-frame ATG codons (Fig. 1). The remainder of the coding sequence (Fig. 1) was obtained by overlap of the sequence containing the downstream *fabG* and *acpP* genes (Rawlings, M. and Cronan, J.E., Jr., submitted for publication). A difficulty was encountered when a full-length *fabD* gene was assembled from the segments isolated in our two laboratories. Although the reassembled gene encoded a protein of the expected size, the expressed protein had no detectable malonyl transacylase activity. This raised the possibility that the DNA sequence (obtained several months earlier) was that of a defective protein. We, therefore, made a minibank of 2.0–2.2 kbp *NcoI* fragments of *E. coli* chromosomal DNA in vector pACYC184 and tested for complementation of the *fabD* lesion in a *recA* derivative of strain LA2-89. *fabD*

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TCCTTGAAGCCTTTGGCGGTGGAATTCAC TGGGGCTCCGCGCTGGTTTCGTTTCTAGGATAAGGATTTAAAC	70
ATG ACG CAA TTT GCA TTT GTG TTC CCT GGA CAG GGT TCT CAA ACC GTT GGA ATG CTG GCT	130
M T Q F A F V F P G Q G S Q T V G M L A	
GAT ATG GCG GCG AGC TAT CCA ATT GTC GAA GAA ACG TTT GCT GAA GCT TCT GCG GCG CTG	190
D M A A S Y P I V E E T F A E A S A A L	
GGC TAC GAC CTG TGG GCG CTG ACC CAG CAG GGG CCA GCT GAA GAA CTG AAT AAA ACC TGG	250
G Y D L W A L T Q Q G P A E E L N K T W	
CAA ACT CAG CCT GCG CTG TTG ACT GCA TCT GTT GCG CTG TAT CGC GTA TGG CAG CAG CAG	310
Q T Q P A L L T A S V A L Y R V W Q Q Q	
GGC GGT AAA GCA CCG GCA ATG ATG GCC GGT CAC AGC CTG GGG GAA TAC TCC GCG CTG GTT	370
G G K A P A M M <u>A G H S</u> L G E Y S A L V	
TGC GCT GGT GTG ATT GAT TTC GCT GAT GCG GTG CGT CTG GTT GAG ATG CGC GGC AAG TTC	430
C A G V I D F A D A V R L V E M R G K F	
ATG CAA GAA GCC GTA CCG GAA GGC ACG GGC GCT ATG GCG GCA ATC ATC GGT CTG GAT GAT	490
M Q E A V P E G T G A M A A I I G L D D	
GCG TCT ATT GCG AAA GCG TGT GAA GAA GCT GCA GAA GGT CAG GTC GTT TCT CCG GTA AAC	550
A S I A K A C E E A A E G Q V V S P V N	
TTT AAC TCT CCG GGA CAG GTG GTT ATT GCC GGT CAT AAA GAA GCG GTT GAG CGT GCT GGC	610
F N S P G Q V V I A G H K E A V E R A G	
GCT GCC TGT AAA GCG GCG GGC GCA AAA CGC GCG CTG CCG TTA CCA GTG AGC GTA CCG TCT	670
A A C K A A G A K R A L P L P V S V P S	
CAC TGT GCG CTG ATG AAA CCA GCA GCC GAC AAA CTG GCA GTA GAA TTA GCG AAA ATC ACC	730
H C A L M K P A A D K L A V E L A K I T	
TTT AAC GCA CCA ACA GTT CCT GTT GTG AAT AAC GTT GAT GTG AAA TGC GAA ACC AAT GGT	790
F N A P T V P V V N N V D V K C E T N G	
GAT GCC ATC CGT GAC GCA CTG GTA CGT CAG TTG TAT AAC CCG GTT CAG TGG ACG AAG TCT	850
D A I R D A L V R Q L Y N P V Q W T K S	
GTT GAG TAC ATG GCA GCG CAA GGC GTA GAA CAT CTC TAT GAA GTC GGC CCG GGC AAA GTG	910
V E Y M A A Q G V E H L Y E V G P G K V	
CTT ACT GGC CTG ACG AAA CGC ATT GTC GAC ACC CAG ACC GCC TCG GCG CTG AAC GAA CCT	970
L T G L T K R I V D T L T A S A L N E P	
TCA GCG ATG GCA GCG GCG CTC GAG CTT TAA AAGAGGAAAAATCATGAATTTTGAAGGAAAAATCGCACTGG	1040
S A M A A A L E L	

Fig. 1. Nucleotide and deduced amino acid sequence of the *fabD* gene. The putative malonyl binding site is highlighted. The *NcoI* sites used for the preparation of a pACYC184 minibank (see text) are upstream (Tsay, J.T., Oh, W., Larson, T.J., Jackowski, S. and Rock, C.O., in press) and downstream (Rawlings, M. and Cronan, J.E., Jr., submitted for publication) of the indicated sequence.

colonies were readily obtained (as expected from the restriction map of the region) and were found to contain much higher levels of malonyl transacylase than LA2-89. Subclones of the *NcoI* segment were sequenced and gave a sequence identical to that found previously (Fig. 1). Thus, the sequence of Fig. 1 is that of the active malonyl transacylase. We believe the lack of activity of the γ 235 clone is due to selection against clones carrying the active gene on a high copy number plasmid (see below). When first subcloned from the γ 235 phage,

these clones clearly complemented the *fabD* lesion of strain LA2-89. However, following several cycles of in vivo amplification, these plasmids failed to complement the *fabD* lesion (although recombinational repair still occurred).

The DNA sequence predicted a protein of 309 amino acids. Given the removal of the N-terminal methionine, this translates to a protein of 32,303 Da. This is somewhat smaller than the SDS gel molecular weight reported for purified malonyl transacylase (35.5 kDa)

[1,2], but matches that obtained for the overproduced protein (see below) and that seen in maxicell and in vitro translation analyses (data not shown). The PI of the predicted sequence (pH 4.9) matches that of the purified protein (pH 4.65) [2], and the deduced amino acid composition very closely matches the composition determined for the purified protein (Table I) [2]. Moreover, the deduced sequence contains an A,G,H,S sequence that matches the composition of a somewhat impure malonyl-labeled peptide isolated by Ruch and Vagelos [3].

3.2. Overproduction of malonyl transacylase

The intact *fabD* gene isolated from chromosomal DNA was placed downstream of the *tac* promoter of pCKR101 to give pKM22. Strains harboring pKM22 overproduced malonyl transacylase activity 4-fold in the absence of induction and 155-fold upon IPTG induction. Overproduction of malonyl transacylase resulted in major accumulation of a 32 kDa protein (Fig. 2).

Modulation of the ratio of malonyl to acetyl precursors has been proposed as a means to regulate fatty acid chain length with increased ratios giving longer chain length fatty acids [9–12]. We, therefore, examined the fatty acid composition of cells overproducing malonyl transacylase. Following induction of a wildtype strain harboring pKM22, the amount of *cis*-vaccenate incorporated into the membrane phospholipids increased and the amount of palmitate incorporated decreased relative to a wild-type strain (Table II).

Table I

Comparison of observed and deduced amino acid compositions

Amino acid	Deduced ^a	Observed ^b
K	14	14.6
H	4	4.3
R	8	8.3
D+N	20	19.2
T	16	14.4
E+Q	37	36.2
P	17	17.6
G	24	23.5
A	54	53.5
V	31	30.1
M	10	11.1
I	9	8.4
L	26	26.9
Y	7	7.6
F	8	9.2
C	5	5.3
W	4	–

^aComposition from DNA sequence (Fig. 1) minus the N-terminal methionine

^bCompositional data of Ruch and Vagelos [2] corrected to 304 residues since tryptophan was not determined.

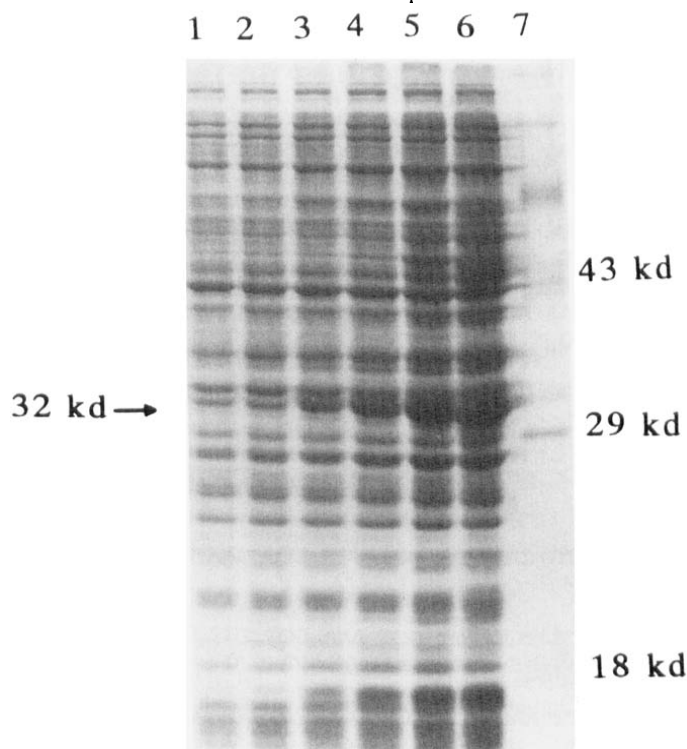


Fig. 2. Accumulation of malonyl transacylase after induction with IPTG. Strain DH5a harboring pKM22 was grown in rich medium supplemented with ampicillin (100 µg/ml). When cultures reached ca. 5×10^8 cells/ml, they were either allowed to incubate for 30 min (–), or IPTG was added to a final concentration of 1 mM (+) and incubation continued for 30 min. At the indicated time points (after induction), samples were taken and lysed in SDS sample buffer. Proteins were analyzed by 12.5% SDS polyacrylamide gel electrophoresis. Lane 1, 0 min; lane 2, 30 min (–); lane 3, 30 min (+); lane 4, 60 min (+); lane 5, 120 min (+); lane 6, 180 min (+); lane 7, molecular weight standards.

3.3. Homology of *E. coli* *FabD* with other enzymes

Genbank was searched with the FASTA program for proteins having significant sequence similarity with *FabD*. Two protein sequences showed greater than 20% identity with *FabD*; an acyltransferase domain of a *Saccharopolyspora* polyketide synthase (30% identity) [13] and a segment of the multifunctional 6-methyl salicylic acid synthase of *Penicillium patulum* (23% identity) [14]. A larger number of sequences similar to the active site region of *FabD* were found including the transacylase (acyltransferase) domain of yeast fatty acid synthase [15,16] where 12 of 16 residues were identical (Fig. 3).

4. DISCUSSION

Malonyl transacylase provides malonyl-ACP, the key intermediate of fatty acid synthesis in organisms (bacteria and plants) having type II (soluble) fatty acid synthases. We have shown that *E. coli* *FabD* is a 308 residue protein with marked sequence similarity to several other proteins having transacylase (acyltransferase) activity. The active site region of *FabD* is

*

FabD	G G K A P A M M A G H S L G E Y S A L V C A G V I D F A D A V R L V E
YSCFAS	L I P A D A T F A G H S L G E Y A A L A S L A D V M S I E S L V E V V
YYLFAS	L V P V D A T F A G H S L G E Y S A L A S L G D V M P I E S L V D V V
PKS	C G A V P S A V I G H S Q G E I A A A V V A G A L S L E D G M R V V A
MSAS	N G I T P Q A V I G H S V G E I A A S V V A G A L S P A E G A L I V T
RATFAS	M G L K P D G I I G H S L G E V A C G Y A D G C L S Q R E A V L A A Y
CHKFAS	A G L Q P D G I L G H S V G E L A C G Y A D N S L S H E E A V L A A Y

Fig. 3. Amino acid similarity of the active site region of FabD to the acyltransferase domains of other enzymes. The active site serine is indicated with an asterisk. YSCFAS, *Saccharomyces cerevisiae* fatty acid synthase; YYLFAS, *Yarrowia lipolytica* fatty acid synthase; PKS, *Saccharopolyspora* polyketide synthase; MSAS, *Penicillium patulum* 6-methyl salicylic acid synthase; RATFAS, rat fatty acid synthase; CHKFAS, chicken fatty acid synthase.

markedly similar to the transacylase domain of several polyfunctional proteins catalyzing fatty acid synthesis and the synthesis of polyketide antibiotics and methyl salicylic acid. Our results strengthen the assignments of these domains and are consistent with other data indicating that these enzyme systems evolved from a common precursor [17].

Modulation of the ratio of acetyl to malonyl precursors has been postulated to regulate fatty acid chain length [9–12]. Increased malonyl/acetyl ratios favor chain elongation in vitro whereas decreased ratios result in shorter chain length. However, this model had not been fully tested in vivo. Harder et al. [4] reported that under conditions of decreased malonyl transacylase activity, *E. coli* synthesizes more shorter chain fatty acids (increased C14:0 relative to C16:0) than a wild-type strain, the effects of a decreased malonyl/acetyl ratio. We report that overproduction of malonyl transacylase has only a minor effect on the fatty acid composition of the *E. coli* phospholipids. Since excess malonyl-CoA and ACP are available in the cytosol of wild-type cells [19,20], we expect that overproduction of malonyl transacylase results in increased levels of malonyl-ACP. The

only change we observed in cells overproducing malonyl transacylase was an increase in the C18:1 (*cis*-vaccenic acid) content of the membrane phospholipids. As expected from previous results, increased C18:1 gave a concomitant decrease in C16:0 (the two acids compete for incorporation into position 1 of the phospholipid backbone) [21]. No increase was seen in C18:0 content nor were any abnormally long fatty acids synthesized. Since such acids are readily incorporated into phospholipids [22], we conclude that up regulation of malonyl transacylase activity cannot regulate acyl chain length in *E. coli*. We attribute the small increase in C18:1 synthesis to stimulation of the activity of 3-ketoacyl-ACP synthase II, the enzyme responsible for elongation of C16:1 to C18:1 [23]. The increased C18:1 synthesis seen upon 150-fold overproduction of malonyl transacylase is much less than that seen upon a 10-fold overproduction of 3-ketoacyl-ACP synthase I [23] or the decrease seen when 3-ketoacyl-ACP synthase III was overproduced (Tsai et al., *J. Biol. Chem.*, in press) indicating that the condensation enzymes (the 3-ketoacyl-ACP synthases) are the key fatty acid synthetic enzymes regulating acyl chain length.

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Table 11

Fatty acid composition of membrane phospholipids

Plasmid (IPTG)	Methyl esters (wt. %)					C18:1/ C16:1
	C14	C16	C16:1	C18	C18:1	
pCKR101 (–)	5.4	33.5	37.9	1.1	22.1	0.58
pCKR101 (+)	5.5	31.4	39.4	1.6	22.1	0.57
pKM22 (–)	3.0	31.4	39.0	1.6	24.4	0.62
pKM22 (+)	5.2	29.4	36.2	1.0	28.2	0.78

The host strain was SJ16, a wild-type strain [18]. Cultures were grown at 37°C in minimal medium supplemented with glycerol (0.4%), vitamin-free casein hydrosylate (0.1%), pantothenate (0.1 µM) and ampicillin (100 mM). When the cultures reached ca. 3×10^8 cells/ml, they were split into two flasks, one containing IPTG (1 mM final concentration) (+) or to a second flask lacking IPTG (–). The cells were harvested 205 min after induction and fatty acid methyl esters were prepared from the phospholipids and analyzed by gas chromatography on a polysilicone capillary column. The C16:1 value includes the small amount of (<8%) of C17 cyclopropane fatty acids formed by post-synthetic modification of the phospholipid-bound chains.

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