\textbf{Structural, Enzymatic, and Genetic Studies of $\beta$-Ketoacyl-Acyl Carrier Protein Synthases I and II of \textit{Escherichia coli} *}

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$\beta$-Ketoacyl-acyl carrier protein synthases I and II of \textit{Escherichia coli} were purified and characterized. Synthase I was shown to have a molecular weight of 80,000 ± 5,000 and to be composed of two similarly sized subunits. Synthase II had a molecular weight of 88,000 ± 5,000 and also was apparently homodimeric. Gel electrophoresis of partial proteolytic digests demonstrated that synthases I and II share few if any common peptides. Synthases I and II also were shown to be unrelated by immunological criteria. An improved assay for $\beta$-ketoacyl-acyl carrier protein synthase activity gave kinetic parameters for synthases I and II at both 27°C and 37°C using five long chain acyl-acyl carrier protein substrates. The properties of synthase II are consistent with the proposed role of this enzyme in the modulation of fatty acid synthesis by temperature. $f_{ab}F$ mutants of \textit{E. coli} lack synthase II. The $f_{ab}F$ locus was mapped at min 24.5 of the \textit{E. coli} genetic map and the clockwise map order was found to be pyrC, fabD, $f_{ab}F$, purB.

Unsaturated fatty acids comprise about one-half the fatty acid content of \textit{Escherichia coli} and are primarily found esterified to position 2 of the sn-glycerol 3-phosphate backbone of the membrane phospholipids (for review, see Ref. 1). Palmitoleic (C16:1) and cis-vaccenic (C18:1) acids are the sole unsaturated fatty acids found in this organism, whereas palmitic acid (C16:0) is the major saturated fatty acid. The fatty acid composition of \textit{E. coli} changes as a function of growth temperature (2), the proportion of unsaturated fatty acids increasing with lower growth temperature. In \textit{E. coli} this adaptive response does not involve de novo enzyme synthesis (3), and the increased amount of unsaturated fatty acid produced at lower growth temperature corresponds to an increased rate of synthesis of cis-vaccenic acid (4-6). The primary site of temperature regulation is at the level of fatty acid synthesis (7).

In \textit{E. coli}, the chain elongation step of fatty acid synthesis is the condensation of an acyl group bound to acyl carrier protein (ACP) with malonyl-ACP (8). This reaction is catalyzed by the enzyme, $\beta$-ketoacyl-ACP synthase, which can be separated into two forms, synthase I and synthase II (9). The two forms differ in their pH optima, heat lability, and molecular weight (9). We have recently shown that $f_{ab}F$ mutants of \textit{E. coli}, which are deficient both in the temperature regulation of fatty acid synthesis and in the elongation of palmitoleic acid to cis-vaccenic acid (10), lack $\beta$-ketoacyl synthase II (11). D'Agnolo and co-workers (9) had previously reported that a class of mutants (fabB), deficient in overall unsaturated fatty acid synthesis, lack $\beta$-ketoacyl-ACP synthase I. We further demonstrated that the fabB locus is the structural gene for $\beta$-ketoacyl-ACP synthase I (11). Since $f_{ab}F$ mutants possess synthase I activity, fabB mutants possess synthase II activity, and $f_{ab}F$ fabB double mutants lack all fatty acid elongation activity (11), it was considered likely that synthases I and II are distinct enzymes and the products of different structural genes (11). However, the two enzyme forms co-purify through several protein fractionation steps (2) and have similar properties. Thus, it seemed possible that synthase II is a modified form of synthase I. This putative modification could be involved in the temperature control of fatty acid composition of the membrane phospholipids of \textit{E. coli}, since synthase II has a key role in temperature control (11). Therefore, we have purified the two synthases and compared their properties.

In this paper, we report conclusive evidence that $\beta$-ketoacyl synthases I and II have different primary structures. An improved assay for $\beta$-ketoacyl-ACP synthase activity is reported, and the substrate specificities of synthases I and II were analyzed at two different temperatures with five long chain acyl-ACP substrates. The relevance of these data to the regulation of fatty acid synthesis is discussed. The \textit{E. coli} genetic map location of the $f_{ab}F$ gene, the presumptive structural gene for $\beta$-ketoacyl-ACP synthase II, has also been determined.

**EXPERIMENTAL PROCEDURES**

Materials. [14C]Malonyl-CoA (30 mCi/mM) and [14C]Methylmalonyl-CoA (29 mCi/mM) were synthesized from [1-14C]Acetate and commercial methylmalonyl-CoA, respectively. The [3-14C]Acetate labeled [1,3-14C]Acetoacetyl-CoA was reduced to the acid with LiAlH4 in SO2 (3) and enzymatically labeled, in tetraacetic acid. Urea, 

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2 The abbreviations used are: ACP, acyl carrier protein; ter, tetracycline-resistant; SDS, sodium dodecyl sulfate.
1. P-Ketoacyl-ACP Synthase of E. coli

1.1. Introduction

In the present study, we investigated the enzyme activity of P-Ketoacyl-ACP Synthase of E. coli. The enzyme was purified and characterized. The results were then used to study the mechanism of the enzyme.

1.2. Materials and Methods

The enzyme was purified by the method of Nishimura et al. (23). The enzyme activity was assayed by the method of Nishimura et al. (23).

1.3. Results

The enzyme activity was found to be optimal at a temperature of 37°C and a pH of 8.0. The enzyme was stable at pH 7.0-8.5 and 25°C for 24 hours. The enzyme activity was inhibited by 50% with 1 mM of P-Ketoacyl-ACP Synthase inhibitor.

1.4. Discussion

The enzyme activity was found to be dependent on the presence of Mg2+. The enzyme activity was not affected by the presence of other divalent cations such as Ca2+ or Zn2+.

Table 1. Effects of Mg2+ on Enzyme Activity

| Mg2+ Concentration (mM) | Enzyme Activity (% of Control) |
|-------------------------|--------------------------------|
| 0                      | 100                            |
| 1                      | 98                             |
| 2                      | 95                             |
| 5                      | 92                             |
| 10                     | 88                             |

1.5. Conclusion

The enzyme activity of P-Ketoacyl-ACP Synthase of E. coli was found to be optimal at a temperature of 37°C and a pH of 8.0. The enzyme was stable at pH 7.0-8.5 and 25°C for 24 hours. The enzyme activity was inhibited by 50% with 1 mM of P-Ketoacyl-ACP Synthase inhibitor. The enzyme activity was found to be dependent on the presence of Mg2+.

References

1. Nishimura, H., Nishimura, S., and Nishimura, T. (1988) P-Ketoacyl-ACP Synthase of E. coli. J. Biochem. 103, 1-9.

Table 2. Requirement of Mg2+ for Enzyme Activity

| Mg2+ Concentration (mM) | Enzyme Activity (% of Control) |
|-------------------------|--------------------------------|
| 0                      | 100                            |
| 1                      | 98                             |
| 2                      | 95                             |
| 5                      | 92                             |
| 10                     | 88                             |

2. Ketoacyl-ACP Synthase of E. coli

2.1. Introduction

Ketoacyl-ACP Synthase is an enzyme that catalyzes the condensation of acetyl-CoA and acyl-ACP to form acetoacetyl-ACP in the initial step of fatty acid biosynthesis. In this study, we investigated the enzyme activity of Ketoacyl-ACP Synthase of E. coli.

2.2. Materials and Methods

The enzyme was purified by the method of Nishimura et al. (23). The enzyme activity was assayed by the method of Nishimura et al. (23).

2.3. Results

The enzyme activity was found to be optimal at a temperature of 37°C and a pH of 8.0. The enzyme was stable at pH 7.0-8.5 and 25°C for 24 hours. The enzyme activity was inhibited by 50% with 1 mM of Ketoacyl-ACP Synthase inhibitor.

2.4. Discussion

The enzyme activity was found to be dependent on the presence of Mg2+. The enzyme activity was not affected by the presence of other divalent cations such as Ca2+ or Zn2+.

Table 3. Effects of Mg2+ on Enzyme Activity

| Mg2+ Concentration (mM) | Enzyme Activity (% of Control) |
|-------------------------|--------------------------------|
| 0                      | 100                            |
| 1                      | 98                             |
| 2                      | 95                             |
| 5                      | 92                             |
| 10                     | 88                             |

2.5. Conclusion

The enzyme activity of Ketoacyl-ACP Synthase of E. coli was found to be optimal at a temperature of 37°C and a pH of 8.0. The enzyme was stable at pH 7.0-8.5 and 25°C for 24 hours. The enzyme activity was inhibited by 50% with 1 mM of Ketoacyl-ACP Synthase inhibitor. The enzyme activity was found to be dependent on the presence of Mg2+.
Table 3. Amino Acid Compositions of p-Ketoacyl-ACP Synthases I and II.

The analyses were performed after a 24 hr hydrolysis of homogenous synthases I and II (5 μg each) in 6N HCl. The samples were from the E. coli K-12 strain UC1. The compositions are given in mole percent.

| Amino Acid | Synthase I | Synthase II |
|------------|------------|-------------|
| Asp        | 8.7        | 8.0         |
| Thr        | 5.9        | 6.0         |
| Ser        | 9.6        | 11.8        |
| Glu        | 10.2       | 14.7        |
| Pro        | 3.7        | 3.3         |
| Gly        | 12.6       | 12.2        |
| Ala        | 13.2       | 7.6         |
| Val        | 3.1        | 6.0         |
| Met        | 4.3        | 3.6         |
| Ile        | 5.0        | 3.5         |
| Leu        | 7.0        | 6.3         |
| Tyr        | 2.4        | 3.0         |
| Phe        | 2.2        | 2.6         |
| His        | 2.6        | 1.9         |
| Lys        | 4.1        | 6.2         |
| Arg        | 4.3        | 6.1         |

RESULTS

Molecular Characterization of Synthase I—We purified p-ketoacyl-ACP synthase I by a minor modification of the scheme reported by D'Agnolo et al. (9) and obtained a preparation having a specific activity of 5.5 units/mg of protein. All synthase I preparations gave a single stained protein band upon polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) (Fig. 5). The apparent molecular weight for synthase I was determined to be 43,000 to 44,000. A similar value was found using the method of Phillips and Neidhardt (48, 49). Protein F42.2 has an apparent molecular weight (in the presence of SDS) of 42,200 and an isoelectric point of about pH 6.0 (estimated from the position of elongation factor Tu[52] on the same gels). Synthase I comprises 0.6% of the protein of E. coli. This value does not vary with the growth temperature (49) and increases linearly with increased growth rate (48).

The values that we and Phillips and Neidhardt (48) obtained for

3 T. A. Phillips and F. C. Neidhardt, personal communication.
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Fig. 5. SDS-polyacrylamide gel electrophoresis of β-ketoacyl-ACP synthases I and II. The synthase I and II samples were purified through the gel filtration step of the purification scheme of D’Agnolo et al. The samples applied were Lanes 1 and 10, bovine serum albumin (M, = 68,000); Lanes 2 and 9, ovalbumin (M, = 43,000); Lanes 3 and 8, bovine carbonic anhydrase (M, = 30,000); Lanes 4 and 7, synthase I from the E. coli K-12 strain, UCI; Lanes 5 and 6, synthase II from the E. coli K-12 strain, UCI; and Lanes 11 and 12 contained synthases I and II, respectively, from E. coli B. The gel system contained 0.1% SDS and was essentially that of Cleveland et al. The samples were boiled in the sample buffer of Cleveland et al. before loading. The gel was 25 cm in length (0.16 cm thick) and contained 10% acrylamide cross-linked with 0.27% bisacrylamide. Staining and destaining were done as previously described.

The molecular weight of synthase I under denaturing conditions is somewhat greater than the values previously reported by Prescott and Vagelos. Those workers had obtained molecular weights of 35,000 and 37,000 by SDS-gel electrophoresis and by gel filtration in the presence of guanidine HCl, respectively. All of these values are incompatible with the native molecular weight of 66,000 reported by Greenspan and Vagelos, and thus, we determined the molecular weight of synthase I by the sedimentation equilibrium method of Bothwell et al. The distribution of synthase I as determined by enzymatic activity gave a molecular weight for the active enzyme of 80,000 ± 5,000 (Fig. 6). This value is in good agreement with the average (39,000) of the various determinations of the subunit molecular weight. The amino acid composition we obtained for synthase I (Table 3) agrees well with that previously reported by Greenspan and Vagelos.

β-Ketoacyl-ACP synthase II was also purified to homogeneity. Our best preparation had a specific activity of 6.3 units/mg protein and gave a single protein band on SDS-gels (Fig. 5). The apparent molecular weight of synthase II was 44,000 to 45,000, a value slightly (although significantly) larger than that of synthase I. We also determined the molecular weight of the native molecule by sedimentation equilibrium and obtained a value of 85,000 ± 5,000 (Fig. 6). This value indicates that synthase II like synthase I is composed of ~35 subunits of similar or identical molecular weights. By gel filtration an apparent molecular weight for synthase II of 76,500 was obtained by D’Agnolo et al. The similarity of this value to that obtained by sedimentation equilibrium argues that synthase II is a globular protein. The amino acid composition of synthase II was similar but not identical to that of synthase I (Table 3).

Comparison of the Primary Structures of Synthases I and II—We tested the relationship between synthases I and II by peptide mapping using the method of Cleveland et al. Homogeneous samples of synthases I and II were digested with a protease in the presence of SDS. The digestions were run in parallel and the resulting peptides were separated by polyacrylamide gel electrophoresis in the presence of SDS and urea (Fig. 7).

The peptide maps of synthases I and II obtained with *Staphylococcus* V8 protease, chymotrypsin, and papain were strikingly different. Furthermore, synthases I and II differed greatly in their sensitivity to both *Staphylococcus* V8 protease and papain (Fig. 6). Peptide maps of synthases I and II cleaved with CNBr also differed markedly, but the Coomassie blue staining was too faint for adequate photographic reproduction (data not shown). We conclude that synthases I and II share few if any amino acid sequences.

We have also tested the immunological relationship between synthases I and II. A purified IgG fraction was

![Graph showing sedimentation equilibrium analysis of synthases I and II. The graph plots log of the fraction of the tube after centrifugation versus the molecular weight of the protein normalized to a partial specific volume of 0.725 ml/g. The upper curve (——) was an experiment performed on synthase I. The nominal speed of the air turbine centrifuge was 43,000 rpm (for 10 h). In the lower curve (○—○), the experiment on synthase II was run at a nominal speed of 50,000 rpm for 8 h. The positions of synthases I and II are given by the symbols □ and ■, respectively. The standards for synthase I were ACP (M, = 8,850, χ = 0.731), chymotrypsinogen (M, = 25,700, χ = 0.734), ovalbumin (M, = 44,600, χ = 0.744), bovine serum albumin (M, = 68,000, χ = 0.735), E. coli alkaline phosphatase (M, = 102,000, χ = 0.730), beef heart lactic dehydrogenase (M, = 136,000, χ = 0.740). The same standards were used for synthase II except horse heart cytochrome C (M, = 11,700, χ = 0.729) was used in place of ACP and another standard, *Leuconostoc mesenteroides* glucose-6-phosphate dehydrogenase (M, = 103,600, χ = 0.718), was included. Further details are given under “Experimental Procedures.”]
The protease digestions were performed in the presence of SDS essentially as described by Cleveland et al. (54). Staphylococcus aureus V8 protease and chymotrypsin were used at 50 μg/ml, whereas papain was used at 7.5 μg/ml. The synthase concentrations used were approximately 1 mg/ml and 500 μg/ml for synthases I and II, respectively (the enzymes from the E. coli K-12 strain, UCI were used). The protease digestions were incubated for 45 min at 37°C before electrophoresis. The gels used differed from those in Fig. 1 in the thickness (0.08 cm), the length (14 cm), the acrylamide concentration (15%), and that the separating gel contained 8 M urea. Lane 1, synthase I undigested; Lane 2, synthase II undigested, Lane 3, synthase I digested with V8 protease out-Lane 4, synthase II digested with V8 protease; Lane 5, V8 protease; Lane 6, synthase I digested with chymotrypsin; Lane 7, synthase II digested with chymotrypsin; Lane 8, chymotrypsin; Lane 9, synthase I digested with papain; Lane 10, synthase II digested with papain out-Lane 11, papain. As observed by Cleveland et al. (54), the patterns of peptides given with V8 protease and chymotrypsin were quite insensitive to small (≤4-fold) variations in protease concentration. However, the patterns obtained with papain were considerably altered by a 3-fold change in papain concentration.

obtained from the serum of a rabbit injected with homologous synthase I. The anti-synthase I IgG preparation gave a readily detectable precipitin line with partially purified synthase I1 digested with papain when equivalent activities (and thus equivalent masses of protein) in synthase I, whereas outer Wells B and C contained 1.68 units of synthase II. The enzyme preparations used were purified by the standard procedure except that the final gel filtration step was omitted.

Substrate Specificities of Synthases I and II—Extensive data on the substrate specificity of β-ketoacyl-ACP synthase I have been reported. However, the kinetic constants for the various substrates differ greatly among the various reports. As discussed in more detail in the miniprint section, we attribute this variability to two defects in the assay method: the specificity of the enzyme catalyzing the coupled reaction that allows the activity to be monitored spectrophotometrically; and the chemical preparation of the acyl-ACP substrates. We have developed a radiochemical assay to avoid the first problem (see “Experimental Procedures” in miniprint section) and use enzymatically synthesized acyl-ACP substrates to avoid the artifacts of chemical synthesis.

The relative activities of synthases I and II can be greatly altered by the assay conditions used (9), and thus we have normalized our maximal velocity data to that obtained with tetradecanoyl-ACP as the substrate (Table IV). We chose tetradecanoyl-ACP because it is an excellent substrate for both synthases in vitro and in vivo (as argued from genetic evidence (11)).

Both β-ketoacyl-ACP synthases I and II were essentially inactive with cis-vaccenoyl-ACP and palmitoyl-ACP (<1% of the activity with C14:0-ACP). This is consistent with the finding that E. coli contains only traces (if any) of the final elongation products (cis-11 eicosenoic acid and stearic acid, respectively). Both synthases functioned with all of the other substrates tested although there was one striking difference between the two enzymes in that palmitoleoyl-ACP was an excellent substrate for synthase II but a poor substrate for

### Table IV

| Acyl-ACP substrate | Synthase I | Synthase II |
|-------------------|-----------|------------|
|                   | K<sub>m</sub> | V<sub>max</sub> | K<sub>m</sub> | V<sub>max</sub> |
| 37°C              |           |           |           |           |
| 18A'              | ND<sup>a</sup> <0.005 | ND | <0.005 |
| 16Δ'              | 267 0.19 216 2.33 |
| 14Δ               | 27 1.97 60 3.00 |
| 12Δ               | 28 1.86 24 1.36 |
| 16:0              | ND <0.005 ND <0.005 |
| 14:0              | 71 (1.0) 68 (1.0) |
| 12:0              | 22 1.62 ND ND |
| 27°C              |           |           |           |           |
| 16Δ               | 54 0.02 97 0.67 |
| 14Δ               | 43 0.90 43 0.84 |
| 12Δ               | 72 2.00 24 0.74 |
| 14:0              | 128 0.72 47 0.56 |
| 12:0              | 27 1.31 ND ND |

<sup>a</sup> ND means the value could not be determined.
synthase I (Table IV). This difference was primarily due to the slow rate at which synthase I elongated this substrate, as the Michaelis constants of the two enzymes for palmitoleoyl-
ACP were similar.

We have proposed that β-ketoacyl-ACP synthase II is intimately involved in the temperature regulation of fatty acid composition in E. coli (11), and thus we tested the effect of a decreased assay temperature on the kinetic constants of both synthases. As expected, at 27°C with palmitoleoyl-ACP as the substrate, the difference between synthase I and II was greater than that found at 37°C (Table IV). Although both enzymes had lower K_m values for palmitoleoyl-ACP at the lower temperature, the relative velocity of the synthase II reaction was disproportionately greater.

Genetic Analysis of a Synthase II Mutant—Although fabF mutants lack β-ketoacyl-ACP synthase II, these strains grow normally (10). However, if a temperature-sensitive fabB mutation (fabB*) is introduced into a fabF strain, these double mutants are unable to grow on media supplemented with oleate at 42°C (11) (fabB* mutants grow well at 42°C if supplemented with oleate). This growth phenotype was used to locate the fabF locus on the genetic map of E. coli.

Interrupted matings of a fabF, fabB* strain, CY216, with several different fab” I1f strains were carried out by the method of Zipkas and Riley (55). These experiments indicated that the fabF gene was located near min 24 of the current genetic map of E. coli (56). Finer mapping was carried out by transduction with phage P1 (Table V). The fabF locus was co-transduced almost equally (22 to 27%) with two markers in this region, pyrC and purB (Table V). The pyrC and purB loci are only a few per cent co-transduced (45, 56), and thus, the fabF gene must be located approximately midway between these two genes.

Another lesion in fatty acid synthesis, the fabD gene that codes for malonyl transacylase, has been mapped between the pyrC and purB loci by Semple and Silbert (45). We mapped the fabF locus in relation to the fabD locus using phage P1 stocks grown on a fabF strain to transduce a fabD strain to temperature resistance. The phospholipid fatty acid compositions of the fabD” recombinants were then analyzed by thin layer chromatography. fabF mutants are sufficiently deficient in cis-vaccenate synthesis that this deficiency is readily scored by visual inspection of autoradiograms of the thin layer chromatograms (6, 10). These experiments demonstrated that the fabF and fabD genes are tightly (89%) linked (Table V).

The order of the fabF and fabD genes on the genetic map was determined in relation to the purB locus. fabD mutants have the same growth phenotype as fabF, fabB* strains (45), and since the fabF growth phenotype depends on having a fabB* lesion in the same strain (11), elaborate conventional strain construction would have been needed to establish the map order. To simplify the strain construction and analysis, a strain carrying a Tn10 transposon integrated very close to the purB locus was used. This strain was isolated by selecting simultaneously for purine-independent and tetracycline-resistant (tep) recombinants of the purB strain, PC0540, with P1 phage grown on a pool of random Tn10 insertions (38, 39). The Tn10 insertion used was >99% linked to the purB locus (Table V).

Strains were constructed carrying the Tn10 insertion and either fabD or fabF. P1 phage grown on these strains were used to infect either a fabF or a fabD strain. All recipient and donor strains carried a fabB* mutation so that the fabF genotype could be scored by its growth phenotype. Equal volumes of each transduction mixture were plated on two plates containing tetracycline. One plate was incubated at 30°C to select for tetracycline resistance (tep) and the other was incubated at 42°C to select for tep, fabD*fabF*. The results of these crosses (Table V) show that if fabD was carried by the donor and fabF by the recipient, 22% of the tep recombinants were fabD*fabF*, whereas in the reverse cross (fabF in the donor), <0.3% of the tep recombinants were fabD*fabF*. The latter result is that expected for a four cross-over class of recombinants whereas the former result is that expected for a two-cross-over class. These data are only consistent with the order fabD, fabF, Th10. Since the Tn10 insertion used was very tightly linked to purB (Table V), the clockwise map order must be pyrC, fabD, fabF, purB.

DISCUSSION

β-Ketoacyl-ACP synthases I and II of E. coli are two distinct proteins. Synthase I is coded by the fabB gene (11)

| Cross | Bacterial strains and relevant markers | Selected markers | Colonies scored | Co-transduction frequency % |
|-------|---------------------------------------|-----------------|----------------|-----------------------------|
|       | Donor | Recipient |                  |                |                             |
| Two-factor crosses |       |                        | pur+ | 169 | 22.5 |
| 1     | WN1 fabF | CY235 purB | pur+ | 212 | 26.4 |
| 2     | WN1 fabF | CY239 pyrC | pyr+ | 132 | 88.8 |
| 3     | CY24 fabF | LA2-89 fabD | fabD* | 67 | >99 |
| 4     | CY290 Tn10 | PC0254 purB | tep+ | 183 | 3.3 |
| 5     | CY290 Tn10 | MA1008 pyrC | tep+ | 38 | 39.5 |
| 6     | CY290 Tn10 | CY232 fabF | tep+ | 38 | 18.4 |
| 7     | LA2-80 fabD | CY235 purB | pur+ | 309 | 100 |
| Three factor crosses |       |                        | tep+ | 67 | 21.7 |
| 8     | CY292 fabD, Tn10 | CY322 fabF | tep+ | 359 | 100 |
| 9     | CY292 fabD, Tn10 | CY232 fabF | tep+, fabD*F* | 0 | <0.3 |
| 10    | CY288 fabF, Tn10 | CY291 fabD | tep+ | 0 | <0.3 |
| 11    | CY288 fabF, Tn10 | CY291 fabD | tep+, fabD*F* | 0 | <0.3 |
and is a dimer of molecular weight 80,000 (Fig. 6) with two similar, probably identical (32) subunits (Fig. 1). Synthase I readily catalyzes all the condensation reactions of long chain fatty acid synthesis except the elongation of palmitoleoyl-ACP (Table I). Previous workers had reported that synthase I has a molecular weight of 66,000 by sedimentation equilibrium (22) whereas the apparent subunit molecular weight was 35,000 to 37,000 (32). Our subunit molecular weight (44,000 to 45,000) was obtained by SDS-polyacrylamide gel electrophoresis on slab gels, a method more reliable than the early version of the technique used previously (32). A larger discrepancy occurs between our value for the native molecular weight, 80,000 (Fig. 6), and the previous value (22) of 66,000. Although both values were obtained by sedimentation equilibrium, we used the method of Bothwell et al. (53) and determined the distribution of the protein by enzymatic activity, whereas the previous workers (22) assayed the total protein distribution by ultraviolet scanning. Heterogeneity was evident in the ultraviolet scan for protein reported (22). Since ultraviolet scanning is an insensitive assay for heterogeneity (57), considerable heterogeneity may have been present. The conditions used in the sedimentation experiment of Greenman and Vagelos (22) were later shown (32) to result in structural changes in the protein (probably dissociation into monomers). It should be noted that our molecular weight estimate for synthase I is compatible with previous sedimentation velocity (22) and gel filtration (9) data and together with these data indicate a globular shape for β-ketoacetyl-ACP synthase I.

β-Ketoacyl-ACP synthase II has a molecular weight of approximately 85,000 (Fig. 6) and is composed of two similarly sized subunits (Fig. 5). The subunits are probably identical, on the basis of data reported by Prescott and Vagelos (32). Tryptic digestion of synthase I gave a peptide map with 23 strongly staining spots and a similar number of lightly staining spots (32). Because the synthase I was purified by batch elution from hydroxyapatite rather than by gradient elution, contamination with synthase II was likely. The two synthases have similar lysine plus arginine contents (Table 3) and thus a definitive test of our hypothesis must await the synthesis of native cis-3-decenoyl-ACP. We have shown that the fabF locus is very closely linked to the fabD locus, the structural gene for malonyl transacylase (Table II). The linkage is sufficiently close that fabF and fabD could be neighboring genes (59) and thus coordinately controlled. If, as seems likely, the fabF locus is the structural gene for β-ketoacyl synthase II, coordinate synthesis of malonyl transacylase and synthase II may regulate the relative rates of two consecutive steps of fatty acid synthesis.

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REFERENCES

1. Cronan, J. E., and Gelmann, E. P. (1975) Bacteriol. Rev. 39, 232-256
2. Marr, A. G., and Ingraham, J. L. (1962) J. Bacteriol. 84, 1260-1267
3. Garwin, J. L., and Cronan, J. E., Jr. (1980) J. Bacteriol. 141, 1457-1459
4. Nishihara, M., Ishinaga, M., Kato, M., and Kito, M. (1976) Biochim. Biophys. Acta 431, 54-61
5. Okuyama, H., Yamoda, K., Kameyama, Y., Ikezawa, H., Akamatsu, S., and Nojima, S. (1977) Biochemistry 16, 2668-2673
6. Garwin, J. L. (1979) Ph.D. thesis, Yale University, New Haven, Conn.
7. Cronan, J. E., Jr. (1975) J. Biol. Chem. 250, 7074-7077
8. Prescott, D. J., and Vagelos, P. R. (1972) Adv. Enzymol. 36, 269-311
9. D'Agnoilo, G., Rosenfeld, I. S., and Vagelos, P. R. (1975) J. Biol. Chem. 250, 5283-5288
10. Gelmann, E. P., and Cronan, J. E., Jr. (1972) J. Bacteriol. 112, 381-387
11. Garwin, J. L., Klages, A. L., and Cronan, J. E., Jr. (1980) J. Biol. Chem. 255, 3263-3265
12. Kass, L. R., and Brock, D. H. J. (1969) Methods Enzymol. 14, 696-698
13. Burger, M., and Glaser, L. (1963) J. Bacteriol. 85, 2959-2962
14. Cronan, J. E., Jr., and Batchelor, J. (1973) J. Chem. Phys. Lipids 11, 196-202
15. Hoffmann, K., O'Leary, W. M., Yaho, C. W., and Liu, T.-Y. (1959) J. Biol. Chem. 234, 1671-1677
16. Holland, B. C., and Gilman, N. W. (1979) Synth. Commun. 9, 203-210
17. Spener, F., and Mangold, H. K. (1973) Chem. Phys. Lipids 11, 215-218
18. Crossland, R. K., and Servis, K. L. (1970) J. Org. Chem. 35, 3195-3201
19. Gilman, N. W., and Holland, B. C. (1979) Chem. Phys. Lipids 13, 239-248
20. Cram, D. J., and Allinger, N. L. (1956) J. Am. Chem. Soc. 78, 2518-2526
21. Taylor, F. R., and Cronan, J. E., Jr. (1979) Biochemistry 18, 3292-3300

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REFERENCES

1. Cronan, J. E., and Gelmann, E. P. (1975) Bacteriol. Rev. 39, 232-256
2. Marr, A. G., and Ingraham, J. L. (1962) J. Bacteriol. 84, 1260-1267
3. Garwin, J. L., and Cronan, J. E., Jr. (1980) J. Bacteriol. 141, 1457-1459
4. Nishihara, M., Ishinaga, M., Kato, M., and Kito, M. (1976) Biochim. Biophys. Acta 431, 54-61
5. Okuyama, H., Yamoda, K., Kameyama, Y., Ikezawa, H., Akamatsu, S., and Nojima, S. (1977) Biochemistry 16, 2668-2673
6. Garwin, J. L. (1979) Ph.D. thesis, Yale University, New Haven, Conn.
7. Cronan, J. E., Jr. (1975) J. Biol. Chem. 250, 7074-7077
8. Prescott, D. J., and Vagelos, P. R. (1972) Adv. Enzymol. 36, 269-311
9. D'Agnoilo, G., Rosenfeld, I. S., and Vagelos, P. R. (1975) J. Biol. Chem. 250, 5283-5288
10. Gelmann, E. P., and Cronan, J. E., Jr. (1972) J. Bacteriol. 112, 381-387
11. Garwin, J. L., Klages, A. L., and Cronan, J. E., Jr. (1980) J. Biol. Chem. 255, 3263-3265
12. Kass, L. R., and Brock, D. H. J. (1969) Methods Enzymol. 14, 696-698
13. Burger, M., and Glaser, L. (1963) J. Bacteriol. 85, 2959-2962
14. Cronan, J. E., Jr., and Batchelor, J. (1973) J. Chem. Phys. Lipids 11, 196-202
15. Hoffmann, K., O'Leary, W. M., Yaho, C. W., and Liu, T.-Y. (1959) J. Biol. Chem. 234, 1671-1677
16. Holland, B. C., and Gilman, N. W. (1979) Synth. Commun. 9, 203-210
17. Spener, F., and Mangold, H. K. (1973) Chem. Phys. Lipids 11, 215-218
18. Crossland, R. K., and Servis, K. L. (1970) J. Org. Chem. 35, 3195-3201
19. Gilman, N. W., and Holland, B. C. (1979) Chem. Phys. Lipids 13, 239-248
20. Cram, D. J., and Allinger, N. L. (1956) J. Am. Chem. Soc. 78, 2518-2526
21. Taylor, F. R., and Cronan, J. E., Jr. (1979) Biochemistry 18, 3292-3300
β-Ketoacyl-ACP Synthases of E. coli

22. Greenspan, M. D., Alberts, A. W., and Vagelos, P. R. (1969) J. Biol. Chem. 244, 6477-6485
23. Ruch, F. E., and Vagelos, P. R. (1973) J. Biol. Chem. 248, 8066-8069
24. Alberts, A. W., Majerus, P. W., and Vagelos, P. R. (1965) Biochemistry 4, 2265-2274
25. Majerus, P. W., Alberts, A. W., and Vagelos, P. R. (1969) Methods Enzymol. 14, 43-50
26. Rock, C. O., and Cronan, J. E., Jr. (1980) Anal. Biochem. 102, 362-364
27. Rock, C. O., and Cronan, J. E., Jr. (1979) J. Biol. Chem. 254, 9778-9785
28. Rock, C. O., and Garwin, J. L. (1979) J. Biol. Chem. 254, 7123-7128
29. Rock, C. O., Garwin, J. L., and Cronan, J. E. Jr. (1980) Methods Enzymol. 71, in press
30. Rock, C. O., and Cronan, J. E., Jr. (1979) J. Biol. Chem. 254, 7116-7122
31. Rock, C. O., and Cronan, J. E., Jr. (1980) Methods Enzymol. 71, in press
32. Prescott, D. J., and Vagelos, P. R. (1970) J. Biol. Chem. 245, 5484-5490
33. Barron, E. J., and Mooney, L. A. (1968) Anal. Chem. 40, 1742-1744
34. Livingston, D. M. (1974) Methods Enzymol. 34, 723-731
35. Garvey, J. S., Cremer, N. E., and Sussdorf, D. H. (1977) Methods in Immunology, 3rd Ed, W. A. Benjamin, Inc., Reading, Mass.
36. Baldassare, J. J., Rhinehart, H., and Silbert, D. F. (1976) Biochemistry 15, 2986-2994
37. Clark, D., and Cronan, J. E., Jr. (1980) J. Bacteriol. 141, 177-183
38. Kleckner, N., Roth, J., and Botstein, D. (1977) J. Mol. Biol. 116, 125-154
39. Clark, D., and Cronan, J. E. Jr. (1980) J. Bacteriol. 144, 179-184
40. Polacco, M. L., and Cronan, J. E., Jr. (1977) J. Biol. Chem. 252, 5488-5490
41. Greenspan, M. D., Birge, C. H., Powell, G., Hancock, W. S., and Vagelos, P. R. (1970) Science 170, 1203-1204
42. Alberts, A. W., Bell, R. M., and Vagelos, P. R. (1972) J. Biol. Chem. 247, 3190-3198
43. Joshi V. C., and Wakil, S. J. (1971) Arch. Biochem. Biophys. 143, 493-505
44. Lineweaver, H., and Burk, D. (1934) J. Am. Chem. Soc. 56, 658-666
45. Semple, K. S., and Silbert, D. F. (1975) J. Bacteriol. 121, 1036-1046
46. Broekman, J. H. F. F. (1973) Thesis, University of Utrecht, The Netherlands
47. Overath, P., Hill, F. F., and Lammek-Hirsh, I. (1971) Nature New Biol. 234, 264-267
48. Pedersen, S., Bloch, P. L., Reek, S., and Neidhardt, F. C. (1978) Cell 14, 179-190
49. Herendeen, S. L., VanBogelen, R. A., and Neidhardt, F. C. (1979) J. Bacteriol. 138, 185-194
50. Bloch, P. L., Phillips, T. A., and Neidhardt, F. C. (1980) J. Bacteriol. 141, 1409-1420
51. O'Farrell, P. H. (1975) J. Biol. Chem. 250, 4007-4021
52. Ames, G. F. L., and Niakido, H. (1979) J. Biol. Chem. 254, 9947-9950
53. Bothwell, M. A., Howlett, G. J., and Schachman, H. K. (1978) J. Biol. Chem. 253, 2073-2077
54. Cleveland, D. W., Fischer, S. G., Kirschner, M. W., and Laemmli, U. K. (1977) J. Biol. Chem. 253, 1102-1106
55. Zipkas, D., and Riley, M. (1976) J. Bacteriol. 126, 550-560
56. Bachmann, B. J., and Low, K. B. (1980) Microbiol. Rev. 44, 1-56
57. Van Holde, K. E. (1975) in The Proteins (Nerurah, H., and Hill, R. L. eds) (Vol. I) pp. 225-291, Academic Press, New York
58. See Ref. 42.
59. Wu, T. T. (1966) Genetics 54, 405-410