Synthesis of Unsaturated Fatty Acids and the Lesion in fab B Mutants*

Ira S. Rosenfeld,‡ Giuliano D’Agnolo,§ and P. Roy Vagelos
From the Washington University School of Medicine, Department of Biological Chemistry, St. Louis, Missouri 63110

SUMMARY
Two classes of Escherichia coli unsaturated fatty acid auxotrophs, which are complementary both in vivo and in vitro, have been utilized to study unsaturated fatty acid synthesis. β-Hydroxydecanoyl thioester dehydrase, which catalyzes the first committed reaction in the unsaturated fatty acid synthetic pathway, is defective in one class, fab A. The defect in the second class, fab B, was unknown. The unsaturated fatty acid synthetic activity defective in fab B extracts was isolated from fab A extracts on the basis of an in vitro complementation assay.

Throughout a 586-fold purification procedure the fab A unsaturated fatty acid synthetic activity was associated with β-ketoacyl acyl carrier protein (ACP) synthetase, suggesting that the two activities are catalyzed by the same protein. Further support for this suggestion derived from the findings that both activities were purified together during the isolation of homogeneous β-ketoacyl-ACP synthetase from wild type E. coli B, both were inhibited to the same extent by iodoacetamide, both were protected against iodoacetamide inhibition by acetyl-ACP, and both were inactivated at 43° at identical rates.

The fact that wild type β-ketoacyl-ACP synthetase stimulated unsaturated fatty acid synthesis by fab B extracts suggested that fab B β-ketoacyl-ACP synthetase is defective in unsaturated fatty acid synthetic activity. Although partially purified fab B β-ketoacyl-ACP synthetase was shown to be defective in unsaturated fatty acid synthetic activity, the mutant enzyme catalyzed normally condensation reactions involving fatty acyl-ACPs that are intermediates in the synthesis of saturated and unsaturated fatty acids. Although the unsaturated fatty acid synthetic activity of β-ketoacyl-ACP synthetase is not understood, these studies demonstrate that this enzyme catalyzes a unique reaction in the synthesis of unsaturated fatty acids.

The synthesis of saturated and unsaturated fatty acids in Escherichia coli proceeds from acetyl coenzyme A and malonyl

* This investigation was supported by Grant GB-5142X from the National Science Foundation and Grant I-R01- HL-10406 from the National Institutes of Health.
‡ National Institutes of Health Postdoctoral Fellow 5-FO2 AM52063-02.
§ On leave from Laboratori di Chimica Biologica, Istituto Superiore di Sanita, Rome, Italy.

The abbreviations used are: ACP, acyl carrier protein.
in fab B strains. (c) The following reactions are catalyzed normally in fab B extracts: dehydration of β-hydroxydecanoyl-ACP to cis-3-decenoyl-ACP (Reaction 1), reduction of cis-5-β-ketodecanoyl-ACP to cis-5-β-hydroxydecanoyl-ACP (Reaction 3), dehydration of this derivative to cis-5-trans-2-dodecadienoyl-ACP (Reaction 4), and reduction of the latter to cis-5-dodecenoyl-ACP (Reaction 5). (d) Incubation of cis-5-β-hydroxydecanoyl-ACP with fatty acid synthetase of wild type or mutant strains gave rise to a similar spectrum of long chain unsaturated fatty acids. (e) Both in vivo and in vitro complementation was demonstrated between the fab A and fab B mutants (16). Abortive transfection with phage P1 was used as the in vivo complementation test, and this test defined the two classes of unsaturated fatty acid auxotrophs denoted as fab A and fab B. In vitro complementation was demonstrated between the two classes by showing that addition of fab A mutant extract to fab B mutant extract permitted normal synthesis of unsaturated fatty acids from acetyl-CoA and malonyl-CoA. In the complementation test fab B mutants contributed the normal β-hydroxydecanoyl thioester dehydrase, while fab A mutants contributed the normal unsaturated fatty acid synthetic activity deficient in fab B mutants.

Since the analysis of purified enzymes and studies of the metabolism of unsaturated fatty acid precursors did not lead to an understanding of the deficiency in the fab B mutants, the unsaturated fatty acid synthetic activity lacking in fab B extracts was isolated from fab A extracts on the basis of the in vitro complementation assay. This paper describes the isolation and partial characterization of this activity.

**EXPERIMENTAL PROCEDURE**

**Materials**—Glucose 6-phosphate dehydrogenase, alcohol dehydrogenase, β-hydroxyacyl-CoA dehydrogenase, malonyl-CoA, glucose-6-P, NADH, NADP+, and streptomycin sulfate were purchased from Sigma. Dithiothreitol was purchased from Calbiochem, while hydroxyapatite and Bio-Gel A 0.5m were obtained from Bio-Rad. Sephadex G-200 and DEAE-Sephadex A-50 were purchased from Pharmacia. DEAE-cellulose 23 and 52 were obtained from Whatman. Diazald was purchased from J. Irich. Silica gel G precoated plates (250 μ) were obtained from Analtech. [2-5]Malonyl-CoA was purchased from New England Nuclear. Fatty acid standards were obtained from Applied Science. All other chemicals and reagents were obtained locally.

ACP was prepared by the method of Majerus et al. (3). Acetyl-ACP, decanoyl-ACP, cis-3-decenoyl-ACP, and cis-5-dodecenoyl-ACP, synthesized as previously described (18, 19) were generously donated by Dr. C. H. Birge. Acetyl-CoA was prepared by the method of Simon and Shemin (20). Malonyl-CoA-ACP transacylase was the generous gift of Dr. F. E. Rudi, Jr.

**Bacterial Strains and Growth Conditions**—The unsaturated fatty acid auxotrophs L010 (fab A) and L020 (fab B) are derivatives of E. coli Hfr 139 and have been previously described (16). Growth conditions have also been reported (12). Cultures were examined for revertants and found to be free of wild type organisms. The temperature-sensitive mutant, UC 204, also of the fab B class, was obtained from Dr. J. E. Cronan, Jr., and grown at 30°C, the permissive temperature for this organism. UC 204 was grown in a liquid medium consisting of tryptone (10 g per liter), NaCl (5 g per liter), and glucose (2 g per liter). E. coli Hfr 139 was grown in the same medium at 37°C. Frozen cells of E. coli B (9 log) were obtained from Grain Processing Corporation, Muscatine, Iowa.

**Analysis of Fatty Acids**—Methyl ester derivatives of fatty acids were prepared by reaction with diazomethane (21). Saturated and unsaturated fatty esters were separated by argen- tination thin layer chromatography. Silicic acid-silver nitrate plates were prepared by dipping the plates in a 10% solution of AgNO₃ in acetone. The plates were dried in air for several minutes and then dried for 1 hour at 120°C. The solvent system used was petrolatum ether diethyl ether (20:1), and approxi mately 1 hour was required for development. A mixture of methyl oleate and methyl palmitate served as standards. Spots were localized following development by spraying with 0.2% 2,7-dichlorofluorescein in ethanol and viewing under ultraviolet light. Radioactivity in the saturated and unsaturated fatty ester fractions was determined in a Packard Tri-Carb liquid scintillation spectrometer model 3380, equipped with a model 544 absolute activity analyzer, using Bray’s solution (22). Gas-liquid radiochromatography of the fatty acids, as methyl esters, has been described (12).

**Fatty Acid Synthesis**—Fatty acid synthetase was prepared from L020 and Hfr 139 by initially dispersing the cells in 0.05 M potassium phosphate buffer, pH 6.8, containing 0.001 M dithiothreitol and 0.001 M EDTA. The cells were ruptured by passage through a French pressure cell at 20,000 p.s.i. and the resulting material was centrifuged at 40,000 × g for 1 hour. The supernatant solution, which contained the synthetase, was divided into numerous small aliquots and stored frozen in liquid nitrogen. It was stable for several months under these conditions.

The in vitro fatty acid synthesis assay used was similar to that of Silbert and Vagelos (17). The typical reaction mixture con-
tained 50 μmoles of potassium phosphate buffer, pH 7.0, 1 μmole of dithiothreitol, 1 μmole of EDTA, 15 μmoles of acetyl-CoA, 30 μmoles of [2-14C]malonyl-CoA (1 μCi per μmole), 1 μmole of NADP+, 2.5 μmoles of glucose-6-P, 2 units of glucose-6-P dehydrogenase, 1 μmole of NADH, 2.5 μmoles of ethanol, 2 units of alcohol dehydrogenase, 25 μg of ACP, and 300 microinits of fatty acid synthetase in a final volume of 0.5 ml. The reaction mixtures were incubated at 37° for 30 min. Reactions were stopped with 0.5 ml of 15% KOH and saponified for 1 hour at 37°. After acidification, the fatty acids were extracted into petroleum ether (b.p. 40–60°). The organic layer was washed once with water and adjusted to 3 ml in graduated conical centrifuge tubes. Aliquots of 0.5 ml were counted to determine the total radioactive fatty acids synthesized. Units of fatty acid synthetic activity are expressed as micromoles of malonyl-CoA incorporated into fatty acids per min.

Fab A (L010) and fab B (L020) mutant extracts catalyze the synthesis of only saturated fatty acids in the fatty acid synthesis assay. Addition of a factor, called unsaturated fatty acid synthetase, present in L010 extract, to L020 extracts. The standard assay for the unsaturated fatty acid synthetic activity was the fatty acid synthesis assay using 300 microinits of L020 fatty acid synthetase and 5 to 50 microinits of L010 unsaturated fatty acid synthetic activity. The fatty acids synthesized in the assay were extracted into petroleum ether, an aliquot was counted as above to determine total fatty acids synthesized, and the remaining petroleum ether solution was allowed to evaporate overnight at room temperature. Unlabeled palmitate and oleic acids were added as internal standards to provide sufficient lipid mass for thin layer chromatography. The fatty acids were converted to methyl esters by treatment with acetyl chloride (21) and chromatographed on silicic acid-silver nitrate thin layer plates. Following chromatography the areas corresponding to saturated and unsaturated fatty acid methyl esters were scraped and the silica gel was placed in liquid scintillation vials and counted after the addition of scintillation fluid. Units of unsaturated fatty acid synthetic activity are expressed as micromoles of malonyl-CoA incorporated into unsaturated fatty acids per min.

**Purification and Assay of β-Ketoacyl-ACP Synthetase—β-Ketoacyl-ACP synthetase from E. coli B was purified to homogeneity by the method of Greenspan et al. (23). Partial purification of the same enzyme from L020 and UC 204 was also accomplished with this method. In these cases the procedure was followed only through the DEAE-cellulose 52 step resulting in approximately a 40-fold purification of the enzymes from these organisms. β-Ketoacyl-ACP synthetase activity was assayed as previously described (23, 24), and units of activity are expressed as micromoles of acetocetetyl-ACP formed per min.

**Purification of Unsaturated Fatty Acid Synthetic Activity from L010—**All operations were performed at 4°. Approximately 130 g of L010 cells were suspended in 400 ml of 0.05 M potassium phosphate buffer, pH 6.8, containing 0.001 M dithiothreitol and 0.001 M EDTA. The cells were ruptured by passage through a French pressure cell at 20,000 p.s.i., and the homogenate was centrifuged at 48,000 × g for 1 hour in a Sorvall RC2-B refrigerated centrifuge. The pellet was discarded, and streptomycin sulfate, 1 mg per mg of protein, was added slowly, with stirring, to the supernatant solution. After stirring for 30 min, the precipitate was removed by centrifugation at 27,000 × g for 15 min and discarded. Solid ammonium sulfate was added to bring the resulting clear solution to 30% saturation. The precipitated material was removed by centrifugation, and the supernatant solution was brought to 75% saturation with additional ammonium sulfate. The precipitated protein, recovered by centrifugation, was dissolved in 250 ml of the initial buffer. Cold hydrochloric acid (0.1 N) was added dropwise, with stirring, until the pH of the solution reached 5.0. The resulting suspension was centrifuged to remove precipitated protein, and the pH of the supernatant solution was brought to 4.0 by the further addition of 0.1 N HCl. After centrifugation, the pellet was suspended in 0.1 M potassium phosphate buffer, pH 6.8, containing 0.001 M dithiothreitol and 0.001 M EDTA. The suspension was stirred overnight at 4° in order to solubilize the unsaturated fatty acid synthetic activity. Insoluble material was removed by centrifugation, and sufficient ammonium sulfate was added to bring the supernatant to 45% saturation. The suspension was then centrifuged, and the resulting supernatant solution was brought to 80% saturation. The precipitated material, removed by centrifugation, was dissolved in a minimal amount of 0.01 M potassium phosphate buffer, pH 7.5, containing 0.001 M 2-mercaptoethanol, 0.001 M EDTA, and 0.06 M KCl. This solution was dialyzed overnight against 40 volumes of the same buffer and loaded onto a DEAE-cellulose 52 column (1 ml of column bed volume per 10 mg of protein) previously equilibrated with the same buffer. The column was washed with several volumes of the equilibrating buffer and subsequently eluted with a linear gradient of 10 column volumes of the same buffer, containing 0.05 to 0.5 M KCl. Fractions (12 ml) were collected, and those containing unsaturated fatty acid synthetic activity were pooled. The solution was concentrated by ultrafiltration in an Amicon cell with a PM-30 membrane. The active material was precipitated from the concentrated solution with ammonium sulfate between 45 and 80% saturation. The precipitate, dissolved in a minimal amount of 0.01 M potassium phosphate buffer, pH 7.5, containing 0.001 M 2-mercaptoethanol and 0.001 M EDTA, was chromatographed on a Bio-Gel A-0.5m column (3 × 75 cm) which was developed with the same buffer. The active fractions were pooled and concentrated as described above. This concentrated material was applied (2 mg of protein per ml of column bed volume) to a DEAE-Sephadex A-50 column equilibrated and developed with a linear gradient of the same buffer composition used for the DEAE-cellulose chromatography. The gradient volume was 20 times that of the column bed volume. After concentration of the active fractions by ultrafiltration, ammonium sulfate was added to 45% saturation. The small precipitate obtained was discarded, and the ammonium sulfate saturation was increased in 5% increments. The material precipitating between 50 and 60% saturation was found to be the most active and was used in all subsequent experiments.

Protein was determined by the microbiuret method of Munkres and Richards (25) or Lowry et al. (26). Disc gel electrophoresis was carried out in a Canalco disc gel apparatus, model 66, with standard 7.5% gels and Tris-glycine buffer, pH 8.3 (27).

**Results**

**In Vitro Fatty Acid Synthesis—**Extracts of the fab B class unsaturated fatty acid auxotrophs such as L020 catalyze primarily the synthesis of saturated fatty acids from acetyl-CoA and malonyl-CoA. This is illustrated in the experiments of Fig. 2A where it is shown that incubation of various concentrations of fatty acid synthetase in the fatty acid synthesis assay gave rise mostly to saturated fatty acids, even at the higher protein concentrations. Addition of extract of the fab A class,
such as LO10, to the fatty acid synthesis reaction mixtures containing a constant amount of LO20 extract, led to the synthesis of unsaturated fatty acids (Fig. 2B). The synthesis of unsaturated fatty acids was proportional to the concentration of LO10 extract under the conditions of the assay. Therefore, the fatty acid synthesis assay, in which the synthesis of unsaturated fatty acids was measured, was utilized during the purification of the unsaturated fatty acid synthetic activity from LO10 extracts.

Purification of Unsaturated Fatty Acid Synthetic Activity—As described under “Experimental Procedure” and summarized in Table I, the unsaturated fatty acid synthetic activity was purified 586-fold with a yield of 28% from LO10 extract. Not shown in Table I are results of some of the preliminary steps (initial ammonium sulfate fractionation and acid precipitation) where the assay was somewhat erratic.

During the course of the purification it became apparent that the unsaturated fatty acid synthetic activity was co-purifying with the \( \beta \)-ketoacyl-ACP synthetase activity of the crude extract. Table I indicates that the ratio of unsaturated fatty acid synthetic activity to \( \beta \)-ketoacyl-ACP synthetase was 0.20 in the crude extract and 0.34 after 586-fold purification. Although the ratio varied between 0.15 and 0.34, it was felt that this variation reflected difficulties with the absolute activities derived from assays carried out at the different stages of purification rather than a real separation of the two activities. The two activities co-chromatographed exactly on any individual chromatography step, as illustrated in Fig. 3, where it is apparent that the two activities were eluted together from DEAE-Sephadex and that the ratio of the two activities was essentially constant across the peak. These experiments suggested that the unsaturated fatty acid synthetic activity is catalyzed by the \( \beta \)-ketoacyl-ACP synthetase.

Properties of Unsaturated Fatty Acid Synthetic Activity and \( \beta \)-Ketoacyl-ACP Synthetase—\( \beta \)-Ketoacyl-ACP synthetase catalyzes the condensation reactions, involving fatty acyl-ACPs

![Fig. 2. In vitro fatty acid synthesis by crude extracts of LO20. A, increasing protein concentrations of LO20 fatty acid synthetase were incubated in the fatty acid synthesis assay. The incubation conditions, saponification, extraction, and separation of the saturated and unsaturated fatty acids were performed as described under “Experimental Procedure.” The ordinate indicates the picomoles of malonyl-CoA incorporated per min into saturated and unsaturated fatty acids. B, to a constant amount of LO20 fatty acid synthetase (200 \( \mu \)g) were added increasing concentrations of crude extracts of LO10. The synthesis of unsaturated fatty acids was determined as in A and is expressed as picomoles of malonyl-CoA incorporated per min. The background of unsaturated fatty acids synthesized by LO20 fatty acid synthetase in the absence of LO10 extract was subtracted from the values plotted.](http://www.jbc.org/)

![Fig. 3. DEAE Sephadex A-50 column chromatography of unsaturated fatty acid synthetic activity and \( \beta \)-ketoacyl-ACP synthetase.](http://www.jbc.org/)

**Table I**

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Unsaturated fatty acid synthetic activity</th>
<th>( \beta )-Ketoacyl-ACP synthetase, specific activity</th>
<th>Ratio of unsaturated fatty acid synthetic activity to ( \beta )-ketoacyl-ACP synthetase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total protein</td>
<td>Total activity</td>
<td>Specific activity</td>
</tr>
<tr>
<td>Crude extract</td>
<td>13,800.0</td>
<td>62.1</td>
<td>0.0045</td>
</tr>
<tr>
<td>DEAE-cellulose 52</td>
<td>104.0</td>
<td>13.4</td>
<td>0.129</td>
</tr>
<tr>
<td>Bio-Gel A-0.5m</td>
<td>31.0</td>
<td>17.7</td>
<td>0.348</td>
</tr>
<tr>
<td>DEAE-Sephadex</td>
<td>14.0</td>
<td>12.7</td>
<td>0.999</td>
</tr>
<tr>
<td>Ammonium sulfate, 50 to 60% saturation</td>
<td>6.8</td>
<td>17.9</td>
<td>2.64</td>
</tr>
</tbody>
</table>

* Enzyme activities were measured as described under “Experimental Procedure.”
The similarity of \( \beta \)-ketoacyl-ACP synthetase and unsaturated fatty acid synthetic activities catalyzed by the 500-fold purified LO10 preparation, several known properties of \( \beta \)-ketoacyl-ACP synthetase were investigated. \( \beta \)-Ketoacyl-ACP synthetase contains a specific cysteine residue that is very sensitive to alkylation by iodoacetamide. Previous experiments have shown that \( \beta \)-ketoacyl-ACP synthetase activity is inhibited when this cysteine is alkylated and that fatty acyl thioester substrates of the enzyme protect the enzyme against inhibition by this alkylating agent (23, 29). The experiments of Fig. 4A demonstrate that \( \beta \)-ketoacyl-ACP synthetase and unsaturated fatty acid synthetic activities were inhibited to the same extent by increasing concentrations of iodoacetamide. With 60 \( \mu \)M iodoacetamide both activities were inhibited 75%. As shown in Fig. 4B, although both activities were inhibited 97% by 200 \( \mu \)M iodoacetamide, addition of acetyl-ACP to the enzyme preparation prior to the iodoacetamide prevented inhibition by the alkylating agent. Both \( \beta \)-ketoacyl-ACP synthetase and unsaturated fatty acid synthetic activities were protected to the same extent at all concentrations of acetyl-ACP tested.

Another similarity between the two activities is illustrated by the heat inactivation experiment shown in Fig. 5. The purified LO10 preparation was heated at 43\( ^\circ \)C for increasing lengths of time, quickly cooled, and then assayed for \( \beta \)-ketoacyl-ACP synthetase and unsaturated fatty acid synthetic activities. The first-order plot shows that the two activities were inactivated at identical rates; the half-time for both was 14 min.

The similarity of \( \beta \)-ketoacyl-ACP synthetase and unsaturated fatty acid synthetic activities in the purified LO10 preparation with respect to inhibition by iodoacetamide, protection against alkylation by acetyl-ACP, and heat inactivation supported the suggestion that both activities are catalyzed by a single protein.

**Purification of \( \beta \)-Ketoacyl-ACP Synthetase from Wild Type E. coli B**—Examination of the purified LO10 preparation by disc gel electrophoresis indicated that the \( \beta \)-ketoacyl-ACP synthetase was only 60 to 70% pure. \( \beta \)-Ketoacyl-ACP synthetase had been purified to homogeneity earlier from E. coli B by means of another procedure (23). Since it was important to compare the \( \beta \)-ketoacyl-ACP synthetase and unsaturated fatty acid synthetic activities in a homogeneous preparation, and since purification of \( \beta \)-ketoacyl ACP synthetase from wild type E. coli, strain B, would exclude the possibility that a peculiar effect of the mutagenesis used to obtain the K12 mutant, LO10, was responsible for the results obtained with the purified LO10 preparation, \( \beta \)-ketoacyl-ACP synthetase of E. coli B was purified to homogeneity by published methods (23, 28). As noted with the LO10 preparation, both \( \beta \)-ketoacyl-ACP synthetase and unsaturated fatty acid synthetic activities were purified together from the E. coli B extract. Fig. 6 shows that they co-chromatographed on Sephadex G-200, and Fig. 7 shows that they co-chromatographed on DEAE-Sephadex. The ratio of the two activities was essentially constant across the peaks in both procedures, and examination of the peak fractions obtained from DEAE-Sephadex indicated the presence of a single protein band. Thus a homogeneous preparation of E. coli B \( \beta \)-ketoacyl-ACP synthetase contained the unsaturated fatty acid synthetic activity.

**Unsaturated Fatty Acid Synthetic Activity of Various \( \beta \)-Ketoacyl-ACP Synthetase Preparations**—The fact that the addition of \( \beta \)-ketoacyl-ACP synthetase of either wild type E. coli B or fab A unsaturated fatty acid auxotroph, LO10, to LO20 fatty acid synthetase permitted the latter to synthesize unsaturated fatty acids suggested that the \( \beta \)-ketoacyl-ACP synthetase of
L020 must be defective in unsaturated fatty acid synthetic activity. Therefore, \( \beta \)-ketoacyl-ACP synthetase was partially purified from L020 extracts, using as the assay the synthesis of acetoacetyl-ACP from acetyl-ACP and malonyl-ACP (23, 24). Fig. 8 demonstrates that, whereas the addition of L010 \( \beta \)-ketoacyl-ACP synthetase to L020 fatty acid synthetase caused the synthesis of unsaturated fatty acids, the addition of L020 \( \beta \)-ketoacyl-ACP synthetase did not have this effect. In order to be sure that the L020 enzyme preparation did not contain an inhibitor of unsaturated fatty acid synthesis, the L010 and L020 \( \beta \)-ketoacyl-ACP synthetase preparations were tested together. As indicated in Fig. 8, when equal units of L010 and L020 \( \beta \)-ketoacyl-ACP synthetase were incubated with L020 fatty acid synthetase, the unsaturated fatty acid synthetic activity noted was that expected from the L010 preparation alone. Therefore, the inactivity of the L020 enzyme preparation was not due to the presence of an inhibitor. These experiments demonstrate that the \( \beta \)-ketoacyl-ACP synthetase of L020 is defective in unsaturated fatty acid synthetic activity.

Also shown in Fig. 8 is the fact that the \( E. \ coli \) B \( \beta \)-ketoacyl-ACP synthetase was more active than the L010 \( \beta \)-ketoacyl-ACP synthetase in promoting unsaturated fatty acid synthesis by the L020 fatty acid synthetase. This difference, which was apparent throughout the purification procedure, is not understood.

**Products of Corrected fab B Fatty Acid Synthetase**—The synthesis of unsaturated fatty acids by the L020 fatty acid synthetase upon the addition of L010 or \( E. \ coli \) B \( \beta \)-ketoacyl-ACP synthetase was monitored only by thin layer chromatography during the protein purification procedures. The availability of highly purified \( \beta \)-ketoacyl-ACP synthetase preparations allowed the identification of the fatty acids synthesized in the reconstituted system. As noted in Fig. 9A, the fatty acid synthetase of wild type \( E. \ coli \) K12 (Hfr 139), the parent of the L010 and L020 unsaturated fatty acid auxotrophs, catalyzed the synthesis of both saturated and unsaturated fatty acids. The unsaturated fatty acids constituted 67% of those synthesized and included octadecenoate and hexadecenoate. Thirty-three percent of the fatty acids synthesized were saturated, mostly hexadecanoate, with smaller amounts of octadecanoate and tetradecanoate. The L020 fatty acid synthetase (Fig. 9B), on the other hand, produced only saturated fatty acids with a chain length distribution similar to that of the wild type. When the L010 \( \beta \)-ketoacyl-ACP synthetase was added to the L020 fatty acid synthetase (Fig. 9C), unsaturated fatty acids were synthesized in addition to the saturated fatty acids. Octadecenoate was the major unsaturated fatty acid synthesized in the experiment of Fig. 9C. However, in other experiments both hexadecenoate and octadecenoate were synthesized; the relationship between the pattern of fatty acids synthesized and the concentration of \( \beta \)-ketoacyl-ACP synthetase will be published elsewhere.

**Temperature-sensitive fab B Mutant**—One of the mutants, UC 204, classified as fab B on the basis of genetic and in vitro results.
Purified preparations of β-ketoacyl-ACP synthetase from LO10 (7.7 units per mg), LO20 (0.30 units per mg), and E. coli B (11 units per mg) were added to a constant amount of LO20 fatty acid synthetase (20 μg) in the fatty acid synthesis assay. The assay, extraction, separation, and counting of the unsaturated fatty acids synthesized are described under “Experimental Procedure.” O--O, LO20 β-ketoacyl-ACP synthetase; O--O, LO10 β-ketoacyl-ACP synthetase; - - -0, LO10 + LO20 β-ketoacyl-ACP synthetases, 50% of each at every concentration indicated; ·--·, E. coli B β-ketoacyl-ACP synthetase.

complementation experiments, is a temperature-sensitive unsaturated fatty acid auxotroph (16). This organism grows normally at 30°, but it requires the presence of unsaturated fatty acids in the growth medium in order to grow at 42°. Since the unsaturated fatty acid synthetic activity of β-ketoacyl-ACP synthetase was shown to be defective in fab β mutants, this mutant presented us with the opportunity to compare the differential thermal inactivation of the two activities of this enzyme. As shown in Fig. 10, when β ketoacylACP synthetase, partially purified from UC 204 extracts, was heated at either 43° or 45°, the unsaturated fatty acid synthetic activity was lost at a faster rate than the β-ketoacyl-ACP synthetase activity. This result contrasts markedly with that obtained with the LO10 β-ketoacylACP synthetase (Fig. 5), which lost both activities in parallel during heat inactivation. The preferential loss at elevated temperatures of the unsaturated fatty acid synthetic activity of UC 204 β-ketoacyl-ACP synthetase explains the specific requirement of this organism for unsaturated fatty acids when it is grown at the restrictive temperature.

Fatty Acyl-ACP Specificity of Mutant β-Ketoacyl-ACP Synthetase—As noted above, fab β unsaturated fatty acid auxotrophs require unsaturated fatty acids for growth because they are specifically defective in the synthesis of unsaturated fatty acids. These mutants synthesize saturated fatty acids normally. It was known from earlier studies of homogeneous β-ketoacyl-ACP synthetase that this enzyme can catalyze all the condensation reactions involved in the synthesis of both saturated and unsaturated fatty acids (14). The enzyme of wild type E. coli has similar affinities for the intermediates of

\[ \text{Acetyl-ACP} \rightarrow \text{Propionyl-ACP} \rightarrow \text{Butyryl-ACP} \rightarrow \text{Octanoyl-ACP} \rightarrow \text{Decanoyl-ACP} \]

The saturated biosynthetic pathway, such as acetyl-ACP and decaenoyl-ACP, and for intermediates of the unsaturated biosynthetic pathway, such as cis-3-decenoyl-ACP and cis-5-decenoyl-ACP. A possible explanation for the fab β mutants involving the β-ketoacyl-ACP synthetase would be a specific loss of affinity of the mutant enzyme for the intermediates of the unsaturated fatty acid biosynthetic pathway. Table II demonstrates that the LO10 β-ketoacyl-ACP synthetase, which promotes unsaturated fatty acid synthesis normally, and LO20 β-ketoacyl-ACP synthetase, which is inactive in unsaturated fatty acid synthesis, have similar affinities for all the fatty acyl-ACPs tested. Most important, the \( K_m \) for cis-5-decenoyl-ACP, the first intermediate in the unsaturated pathway that undergoes condensation with malonyl-ACP (Fig. 1, Reaction 2), is essentially the same with the two enzyme preparations. Thus, although LO20 β-ketoacyl-ACP synthetase is defective in a specific function which renders it inactive in the synthesis of unsat-
The experiments discussed above suggest that the defect in the fab B unsaturated fatty acid auxotrophs is localized to the \( \beta \)-ketoacyl-ACP synthetase. This conclusion is based upon two major observations. First, addition of homogeneous wild type \( \beta \)-ketoacyl-ACP synthetase to mutant (fab B) fatty acid synthetase restored the ability of the mutant fatty acid synthetase to synthesize unsaturated fatty acids. Secondly, \( \beta \)-ketoacyl-ACP synthetase, partially purified from fab B mutant extract, did not stimulate unsaturated fatty acid synthesis by the fab B fatty acid synthetase; therefore, the fab B \( \beta \)-ketoacyl-ACP synthetase was defective in unsaturated fatty acid synthetic activity. It is conceivable that the preparations of wild type \( \beta \)-ketoacyl-ACP synthetase examined contained another protein specifically required for unsaturated fatty acid synthesis by the mutant fatty acid synthetase. However, the similarities in response of the \( \beta \)-ketoacyl-ACP synthetase and unsaturated fatty acid synthetic activities of the purified preparation to inhibition by iodoacetamide, protection against iodoacetamide inhibition by acetyl-ACP, and heat denaturation indicate that both activities are catalyzed by a single protein.

The nature of the unsaturated fatty acid synthetic activity of the \( \beta \)-ketoacyl ACP synthetase, as defined by the fab B mutation, is not understood. \( \beta \)-Ketoacyl-ACP synthetase catalyzes the condensation reactions of both saturated and unsaturated fatty acid biosynthetic pathways (12, 14). Thus it was expected that the defective \( \beta \)-ketoacyl-ACP synthetase of fab B mutants would be inactive in condensation reactions involving intermediates of the unsaturated pathway. Although pure mutant enzyme was not available, analysis of a partially purified preparation indicated that the mutant enzyme catalyzed condensation reactions with unsaturated as well as saturated fatty acid biosynthetic intermediates. Thus, although the fab B \( \beta \)-ketoacyl-ACP synthetase is defective in unsaturated fatty acid synthetic activity, in vitro it functioned normally in all the condensation reactions involved in the synthesis of unsaturated fatty acids. It appears, therefore, that the unsaturated fatty acid synthetase activity in the fab B fatty acid synthetase may be a separate and independent activity of \( \beta \)-ketoacyl-ACP synthetase.

At this time we cannot explain the unsaturated fatty acid synthetic activity of \( \beta \)-ketoacyl-ACP synthetase. Although this activity might represent a highly specific enzymatic step of the unsaturated pathway that has not yet been tested, another possibility is that it represents an effector function of \( \beta \)-ketoacyl-ACP synthetase. Bloch and his coworkers (2, 8-11) have studied the \( \beta \)-hydroxydecanoyl thioester dehydrase which catalyzes the dehydration of \( \beta \)-hydroxydecanoyl-ACP to form cis-3-decenoyl-ACP, the first committed reaction in the unsaturated pathway (Fig. 1, Reaction 1). Although in vivo this enzyme functions specifically in the formation of cis-3-decenoyl-ACP, a number of studies have shown that the purified enzyme produces primarily the trans-2-decenoyl thioester which is an intermediate in the synthesis of saturated fatty acids (81).

If \( \beta \)-ketoacyl-ACP synthetase interacted with \( \beta \)-hydroxydecanoyl thioester dehydrase, causing the latter to catalyze the formation of cis-3 rather than trans-2-decenoyl-ACP from \( \beta \)-hydroxydecanoyl-ACP, then loss of this interaction would lead to a specific loss of the ability to synthesize unsaturated fatty acids. Experiments, attempting to demonstrate an effector function of \( \beta \)-ketoacyl-ACP synthetase, are in progress.

Acknowledgments—We wish to thank Dr. Claire H. Birge and A. W. Alberts for their many helpful discussions and suggestions.
during the course of this work, and Mary Leadbetter for her excellent technical assistance. We also wish to thank Dr. D. J. Prescott for the generous gift of purified E. coli B β-ketacyl-ACP synthetase.

REFERENCES

Synthesis of Unsaturated Fatty Acids and the Lesion in fab B Mutants
Ira S. Rosenfeld, Giuliano D'Agnolo and P. Roy Vagelos


Access the most updated version of this article at http://www.jbc.org/content/248/7/2452

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/248/7/2452.full.html#ref-list-1