Enzymatic Synthesis of Cyclopropane Fatty Acids Catalyzed by Bacterial Extracts*

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The pioneering studies of Hofmann and co-workers on the lipids of lactobacilli led to the discovery of a 19 carbon fatty acid containing a cyclopropane ring (1-5). This acid was characterized as cis-11,12-methylenecadecanoic acid and was given the trivial name, lactobacillic acid. A 17 carbon fatty acid from Escherichia coli was described by Dauchy and As- selineau (6) and characterized as cis-9,10-methylenhexadecanoic acid by Kaneshiro and Marr (7). Whole cell experiments demonstrated the importance of the methyl group of methionine as a 1 carbon unit for the conversion of 16 and 18 carbon monounsaturated fatty acids to 17 and 19 carbon cyclopropane acids, respectively (4, 8-10).

We wish to report our studies on the enzymatic synthesis of cyclopropane fatty acids catalyzed by cell-free extracts prepared from the gram-negative aerobe, Serratia marcescens, and the gram-positive anaerobe, Clostridium butyricum. A preliminary account has appeared (11).

EXPERIMENTAL PROCEDURE

Methods

Growth of Cells—Serratia marcescens, ATCC 8195, was grown at 30° on a medium containing 2% glucose, 0.5% peptone, 0.3% beef extract, and salts (12). Clostridium butyricum, ATCC 6015, was grown at 37° on synthetic media which were flushed with N₂ and sealed before incubation (13). Cells were harvested in the log phase of growth by centrifugation and washed once with water or 1.70 KCl. Cells were stored at -20°.

Preparation of Cell-free Extracts—All operations were carried out at 0-5° unless otherwise noted. Suspensions of S. marcescens (20 to 30 g, wet weight, per 100 ml) in 0.1 M phosphate, pH 7.0, were disrupted in a French pressure cell (American Instrument Company, Silver Spring, Maryland). Whole cells and large particles were removed by centrifugation at 18,000 × g for 30 minutes and discarded. The supernatant solution obtained by centrifugation at 100,000 × g for 1½ hours, and the small pellet was discarded. The pH of the clear, yellow-green supernatant solution was adjusted to neutrality with 1 N NaOH, and small aliquots were frozen. Enzymatic activity was stable for at least 1 month under these conditions.

Frozen extracts were used directly after thawing or were dialyzed against 100 volumes of 0.04 M Tris, pH 7.0, for 5 to 10 hours on a rocker dialyzer. Protein content of the extracts was determined by the method of Lowry et al. (15), with bovine serum albumin used as a standard.

Enzymatic Assay of Cyclopropane Acid Synthesis—Bacterial extracts were incubated with labeled S-adenosylmethionine and other additions as noted. NaCN was added to S. marcescens extracts to inhibit the S-adenosylmethionine decarboxylase reaction. Most incubations were conducted at 30° or 37°. Reactions were terminated by addition of an equal volume of methanol and 0.2 volume of 85% potassium hydroxide. These solutions were warmed for 15 minutes to achieve saponification. Early experiments showed that negligible amounts of radioactive materials were extractable in a nonsaponifiable fraction, and this step was therefore omitted in most experiments. Alkaline solutions were acidified to pH 1 by the addition of 6 N HCl. Fatty acids were extracted with three successive 2-ml portions of ether. The combined ether extracts were washed with 2 ml of 2% KCl twice, and aliquots were taken for liquid scintillation counting in a Packard Tri-Carb instrument (Packard Instrument Company, Inc., La Grange, Illinois). Aliquots of fatty acids to be assayed by gas-liquid chromatography were converted to their methyl esters with freshly distilled diazomethane generated from N-methylnitrosourea. Gas-liquid chromatographic analysis of fatty acid esters was performed with a Research Specialties Company (Richmond, California) instrument equipped with an ionization detector and a 6-foot column packed with 10% diethylene glycol succinate on Chromosorb W. The column was operated at 165-185° with an argon flow rate of 40 to 70 ml per minute. Radioactive fatty acid esters were trapped at the outlet of the column in glass U-tubes cooled by

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1 Cyclopropane fatty acid synthesis, as applied to cell-free systems, denotes synthesis of a cyclopropane ring on a preformed carbon chain, not synthesis de novo of the entire fatty acid.
the evaporation of methylene chloride from a cotton wad. For counting, fatty acid esters were rinsed with toluene scintillator fluid from the collection tube into vials. Fatty acid composition was calculated from peak areas obtained by the method of Carroll (16). Retention times were compared with those of standard fatty acid methyl esters (described below).

Column and Thin Layer Chromatography—Chromatographic separation of lipids, shown in Figs. 1 and 2, was accomplished by the column chromatography method of Wren (17), with Mallinckrodt No. 2847 silicic acid which had been previously washed and sieved to 100 to 200 mesh, or Unisil (Clarkson Chemical Company, Williamsport, Pennsylvania). Lipid phosphorus was determined after digestion with 5 N H2SO4 (18). Lipids were also chromatographed on thin layers of silicic acid with techniques and solvent systems of Wagner, Horhammer, and Wolff (19). Lipids were detected by exposure to iodine vapors, followed by spraying with starch. After the spots were marked, a ninhydrin spray (20) was used to identify lipids with free amino groups. Radioautograms of thin layer plates were made by sandwiching Eastman Kodak "no screen" x-ray film between the chromatogram and a matching glass plate.

Results

Nature of 1 Carbon Donor for Synthesis of Cyclopropane Ring—Both methionine and formate have been shown to provide the methylene bridge of lactobacillic acid in whole cells (4). In examining this reaction in bacterial extracts it was first necessary to determine more exactly the identity of the ultimate 1 carbon donor. The results of experiments designed to clarify this point are shown in Table I. Of the 1 carbon donors tested, only serine and S-adenosylhomocysteine served as precursors of fatty acids. The incorporation of serine was not always observed, and when it was, it was invariably lower than the incorporation of S-adenosylmethionine. It should be emphasized that in these experiments the fatty acids which became labeled were not further identified. The serine incorporation could have resulted from prior conversion to acetyl-CoA, followed by random incorporation into fatty acid chains. Indeed, such a result was observed when bacterial cells were grown in the presence of 3-C14-serine. Large scale incubations of extract with labeled S-adenosylmethionine afforded sufficient radioactive fatty acids for gas-liquid chromatographic analysis of the methyl esters (Table II). It can be seen that 90% of the radioactive fatty acid esters are found in the chromatographic effluents corresponding to cyclopropane fatty acid esters.

The experiments recorded in Table I also demonstrate that methionine which has not been activated to the adenosyl derivative is not a suitable substrate for this reaction in vitro.

Synthetic and Commercial Lipoaid—Fatty acids used as standards were purchased from Applied Science Laboratories, Inc., State College, Pennsylvania. Cyclopropane fatty acids of 17 and 19 carbon atoms were synthesized from palmitoleic acid and oleic acid by the method of Simmons and Smith (24). Commercial phospholipids used as standards were obtained from Mann Research Laboratories, New York 6, New York, and Fluka A.G., Chemische Fabrik, Buchs, Switzerland. Phospholipid preparations were examined by thin layer chromatography. No preparation was found to be homogeneous, but comparison of several different samples indicated that the major component of each was that claimed by the vendor. All phosphatidylylethanolamine samples were compared chromatographically with a synthetic sample of dimyristylphosphatidylethanolamine kindly provided by Dr. Erich Baer.

S-Adenosylmethionine and S-Adenosylhomocysteine—S-Adenosyl-l-methionine, both labeled and unlabeled, was prepared either from yeast according to Schlenk, Dainko, and Stanford (25) or synthesized enzymatically by the method of Cantoni (26). The product was purified by the chromatographic procedure of Mudd (27). Purified S-adenosylmethionine acetate gave a single radioactive and ninhydrin-positive spot in the paper chromatographic system, ethanol-acetic acid-water, 64:1:35 (28). Small aliquots of this material were stored at −20° at pH 5. Under these conditions, the compound is stable for many months. S-Adenosyl-l-homocysteine was made by the procedure of Sakami (29). All radioactive compounds used in these experiments were purchased from New England Nuclear Corporation, 675 Albany Street, Boston 15, Massachusetts.

Materials

S. marcescens "Unsaturated" Lipids—Total lipids were extracted from S. marcescens cells harvested in the log phase of growth, and the extracts were washed by the procedure of these workers. Aqueous dispersions of lipids were prepared by either sonication or by the dialysis technique of Fleisher and Klouwen (22). Micellar dispersions of S. marcescens lipids prepared by the dialysis technique were subjected to thin layer chromatography. Three phospholipid spots appeared on staining with iodine: a strong spot (ninhydrin-positive, Rf 0.53) corresponding to phosphatidylethanolamine, and two weaker, ninhydrin-negative spots (Rf 0.79 and 0.39). No neutral lipids or free fatty acids were observed. The S. marcescens "unsaturated" lipids had the following fatty acid composition as determined by gas-liquid chromatography: 16:0, 45%; 17:0, 17%; 17:1, 23%; 18:1, 11%; 19:0, 4%.

S. marcescens "Saturated" Lipids—Total lipids were extracted as described above from S. marcescens cells harvested in the stationary phase of growth. They were likewise washed and dispersed. The component lipids were similar to the S. marcescens "unsaturated" lipid preparation; phosphatidylethanolamine was the major constituent. The fatty acid composition was: 16:0, 49%; 17:0, 41%; 19:0, 9%.

Palmitoleyl-CoA—The coenzyme A ester of palmitoleic acid was prepared by the method of Goldman and Vagelos (23).

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Table I

Survey of substrates for synthesis of fatty acids catalyzed by soluble extracts of S. marcescens

Each reaction in Experiment 1 contained, in a final volume of 0.5 ml: radioactive substrate, 50 pmol (100,000 d.p.m.); NaCN, 1 µmole; phosphate, pH 7.0, 15 to 30 µmole; extract, 1 mg of protein. Reaction time, 6 minutes at 37°. In Experiment 2, each reaction contained, in 0.5-ml volume: uniformly labeled l-serine-C₁⁴, 15 µmole (192,000 d.p.m.); AMe-CH₃-H₃, 5 µmole (150,000 d.p.m.); extract, 2.5 mg of protein; buffer and NaCN, similar to Experiment 1. Reaction time, 10 minutes at 37°. Experiment 3: AMe-CH₃-H₃ or l-methionine-CH₃-H₃, 5 µmole (129,000 d.p.m.); extract, 1 mg of protein; buffer and NaCN, similar to Experiment 1. Reaction time, 10 minutes at 37°. Incorporations into the crude fatty acid fraction, isolated as described in the text, were measured.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Incorporation into fatty acids d.p.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>AMe-CH₃-C₁⁴</td>
</tr>
<tr>
<td></td>
<td>Serine-3-C₁⁴</td>
</tr>
<tr>
<td></td>
<td>Formate-C₁⁴</td>
</tr>
<tr>
<td></td>
<td>Formaldehyde-O₁⁴</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>AMe-CH₃-H₃</td>
</tr>
<tr>
<td></td>
<td>Uniformly labeled serine-C₁⁴</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>AMe-CH₃-H₃</td>
</tr>
<tr>
<td></td>
<td>Methionine-CH₃-H₃</td>
</tr>
</tbody>
</table>

* The abbreviation used in the tables and figures is: AMe, S-adenosylmethionine.

Table II

Incorporation of S-adenosyl-L-methionine-methyl-H₃ into cyclopropane fatty acids catalyzed by soluble extracts of S. marcescens

The reaction mixture, contained in a volume of 0.5 ml: NaCN, 1 µmole; phosphate buffer, pH 7.0, 30 µmole; AMe-CH₃-H₃ (1.3 × 10⁴ d.p.m.); 47 µmole; soluble extract, 4.5 mg of protein. Assay time, 10 minutes at 37°. Radioactive fatty acids were isolated after saponification, extraction, and washing. They were converted to methyl esters and separated by gas-liquid chromatography.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Radioactivity incorporated d.p.m.</th>
<th>Relative percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0-16:1</td>
<td>1,100</td>
<td>1.9</td>
</tr>
<tr>
<td>17:0</td>
<td>47,700</td>
<td>82</td>
</tr>
<tr>
<td>18:0-18:1</td>
<td>4,000</td>
<td>6.9</td>
</tr>
<tr>
<td>19:0</td>
<td>5,500</td>
<td>9.5</td>
</tr>
</tbody>
</table>

* The order of emergence of fatty acid methyl esters from the column, as determined by comparison with standards, is: 14:0, 16:0, 10:1, 17:0, 18:0, 18:1, 19:0. Recovery of radioactivity from the column was 76%.

Addition | Radioactivity incorporated into fatty acids Cypompropane acids formed |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>S. marcescens &quot;unsaturated&quot; lipid, 3 mg</td>
<td>3090 97</td>
</tr>
<tr>
<td>S. marcescens &quot;saturated&quot; lipid, 3 mg</td>
<td>253 7</td>
</tr>
<tr>
<td>Palmitoleyl-CoA, 1.0 µmole</td>
<td>0 0</td>
</tr>
<tr>
<td>Palmitoleic acid, 4.5 µmole</td>
<td>29 1</td>
</tr>
<tr>
<td>Synthetic 22-dipalmitoylphosphatidylethanolamine, 9 µmole*</td>
<td>34 1</td>
</tr>
</tbody>
</table>

* This material failed to give a suitable aqueous dispersion, in accord with the observations of Fleisher and Kliewen (22).
esterified cyclopropane fatty acids. For identification of the actual enzymatic product, reaction mixtures were extracted with chloroform-methanol, washed, and then chromatographed on columns of silicic acid. Fractions were collected and assayed for radioactivity and for lipid phosphorus. Figs. 1 and 2 show the results of reactions catalyzed by S. marcescens extract and by C. butyricum extract. In each case, radioactivity incorpo-
ratated into cyclopropane acids is chromatographically coincident with lipid phosphorus. Material contained in a first peak from the reaction catalyzed by the S. marcescens extract did not contain radioactive fatty acids and was not investigated further.

Further identification of the reaction product from the experiment with C. butyricum extract was achieved by thin layer chromatography and radioautography of an aliquot of the labeled phospholipid product. Fig. 3 shows a montage produced by laying a tracing of the spots developed on a thin layer plate with iodine vapors over the radioautogram and photographing the result. The dark spot represents the radioactive reaction product, which is coincident with a lipid spot. Subsequent spraying of the plate with ninhydrin gave one spot coincident with the reaction product and parallel to the only ninhydrin-positive spot in the synthetic phosphatidylethanolamine.

When purified radioactive phospholipid from the silicic acid column was saponified, all of the radioactive material was recovered in the fatty acid fraction. Conversion to the methyl esters and separation by gas-liquid chromatography confirmed that at least 90% of the radioactive fatty acid esters were indeed 17 and 19 carbon cyclopropane fatty acid esters. Table IV presents data for the reaction product synthesized by the C. butyricum extract.
TABLE IV
Identification of radioactive fatty acids from product of reaction catalyzed by C. butyricum extract

An aliquot (23,200 d.p.m.) of fatty acid methyl esters from the lipid purified by silicic acid chromatography was analyzed by gas-liquid chromatography, and the peaks were collected and counted.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Cn</th>
<th>Radioactivity recovered*</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0-16:1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>17: Cy</td>
<td>5,020</td>
<td>26.2</td>
</tr>
<tr>
<td>18:0-18:1</td>
<td>230</td>
<td>1.2</td>
</tr>
<tr>
<td>19: Cy</td>
<td>12,700</td>
<td>66.1</td>
</tr>
<tr>
<td>&gt;19: Cy</td>
<td>1,190</td>
<td>6.2</td>
</tr>
</tbody>
</table>

* Recovery of C14 from the column was 82.5%.

Properties of Enzymatic Synthesis of Cyclopropane Fatty Acids—The data presented in Fig. 4 describe several aspects of the cyclopropane fatty acid-synthesizing activity present in soluble extracts of C. butyricum. It is apparent that the rate of synthesis is linear over a broad range of protein concentrations (Fig. 4A). The synthesis of cyclopropane fatty acids continues for at least 3 hours (Fig. 4C), indicating the stability of S-adenosylmethionine in this system. A decrease of rate is consistently observed between 30 and 60 minutes. Essentially no reaction occurs in the absence of added lipid. The dependence of cyclopropane acid formation on lipid and S-adenosylmethionine is given in Fig. 4, B and D. Fig. 5 shows the dependence of the enzymatic reaction on pH. A similar curve was obtained with the Serratia system except that the optimum was broader. Extracts from both organisms retained full activity after dialysis against pH 7 buffers for 5 to 10 hours.

A number of reports have appeared which describe the methylation of the amino group of phosphatidylethanolamine giving rise ultimately to phosphatidylethanolamine (30-32). This system from animal liver is inhibited by adenosylhomocysteine (31), sulfhydryl reagents (31, 32), and competitive substrates such as mercaptoethanol (32). Therefore, it was of interest to test the present system in this regard. The data summarized in Table V show p-hydroxymercaptoenzoate inhibition which is reversed by guest on August 28, 2017 http://www.jbc.org/ Downloaded from

![Fig. 4. Properties of cyclopropane fatty acid synthesis by soluble extracts of C. butyricum. Reaction components and conditions as follows: A, AMe-CH3-H3, 760 mpmoles (74,000 d.p.m.); S. marcescens “unsaturated” lipid, 3 μmoles of lipid phosphorus; extract, 0 to 16 mg of protein; Tris buffer, pH 7.0, 10 to 81 μmoles. Final volume, 1.0 ml. Reaction time, 1 hour at 30°. B, AMe-CH3-H3, 760 mpmoles (74,000 d.p.m.); S. marcescens “unsaturated” lipid, 3 to 12 μmoles of lipid phosphorus; extract, 6 μg of protein; Tris buffer, pH 7.0, 21 to 51 μmoles. Final volume, 1.0 ml. Reaction time, 1 hour at 30°. C, AMe-CH3-H3, 760 mpmoles (77,000 d.p.m.); S. marcescens “unsaturated” lipid, 4.5 μmoles of lipid phosphorus; extract, 6 mg of protein; Tris buffer, pH 7.0, 15 to 20 μmoles; extract, 2 mg of protein; added component as indicated. Volume, 0.5 ml. Reactions catalyzed by C. butyricum extract contained: AMe-CH3-H3, 5 μmoles (129,000 d.p.m.); NaCN, 1 μmole; phosphate, pH 7.0, 15 to 20 μmoles; extract, 2 mg of protein; added component as indicated. Reaction time, 1 hour at 30°. D, AMe-CH3-H3, 5 μmoles (129,000 d.p.m.); S. marcescens lipid, 2 mg; extract, 4.5 μg of protein; Tris, pH 7.0, 40 to 60 μmoles. Final volume, 1.0 ml. Reaction time, 60 minutes at 30°.

![Fig. 5. Dependence of cyclopropane fatty acid synthesis catalyzed by C. butyricum extract on pH. Each tube contained dialed extract, 4 mg; S. marcescens “unsaturated” lipid, 3 μmoles of lipid phosphorus; AMe-CH3-H3, 51 mpmoles (77,000 d.p.m.); 0.1 M buffer, 100 μmoles. Final volume was 1.38 ml. Reaction time, 30 minutes at 30°.

TABLE V
Effect of potential inhibitors on cyclopropane fatty acid synthesis

<table>
<thead>
<tr>
<th>Addition</th>
<th>Incorporation into fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. marcescens</td>
<td>C. butyricum</td>
</tr>
<tr>
<td>4900                     1500</td>
<td></td>
</tr>
<tr>
<td>580                      12</td>
<td></td>
</tr>
<tr>
<td>4380                     1930</td>
<td></td>
</tr>
<tr>
<td>6900                     953</td>
<td></td>
</tr>
<tr>
<td>5050                     1820</td>
<td></td>
</tr>
</tbody>
</table>

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by cysteine. Iodoacetate proved to be slightly inhibitory to the reaction catalyzed by C. butyricum extracts, but stimulatory to the S. marcescens reaction.

**DISCUSSION**

The principal features of the synthesis of cyclopropane fatty acids have been defined by studies with growing bacterial cultures. The origin of the methylene bridge from 1 carbon donor (formate or the methyl group of methionine) has been clearly established by Liu and Hofmann (4). In early stages of growth, typical phospholipids, e.g., phosphatidylethanolamine, contain unsaturated fatty acids, whereas in late stages they contain only saturated and cyclopropane acids. Therefore, either fatty acids must be cleaved from phospholipids, converted to cyclopropane acids, and reincorporated into phospholipids, or the synthesis of cyclopropane acids must require intact phospholipid as substrate (10). This is a particularly important point in the case of gram-negative bacteria like Escherichia coli (33) or S. marcescens, in which phosphatidylethanolamine is the major lipid material of the cell.

Clostridium butyricum produces cyclopropane acids (34), but these form a smaller proportion of the total fatty acids than they do in S. marcescens. Furthermore, it does not appear that the unsaturated acids are completely converted to cyclopropane acids even in the stationary phase of growth. The lipid pattern of C. butyricum is more complex than that of S. marcescens. All of these factors may contribute to some unknown way, to the differences in behavior of the two enzyme systems with regard to endogenous and exogenous lipid substrates.

Both extracts contain endogenous lipids. Examination of the amount of endogenous lipid in the Clostridium extracts (30 μg per mg of protein) and of its content of unsaturated fatty acids (18:1 = 2 μg per mg of protein) indicated that it contained less than the amount of octadecenoic acid necessary to account for the synthesis of methyleneoctadecanoic acid observed under the most favorable conditions of stimulation by exogenous lipid. This is the most cogent evidence that the exogenous lipid is indeed serving as a substrate for cyclopropane fatty acid synthesis. The possibility that the fatty acids of the added lipids are cleaved from the molecule, converted to cyclopropane acids, and then added back to give the observed product, phosphatidylethanolamine, cannot be ruled out on the basis of these experiments. If this is the case, however, the intermediate steps cannot involve free unsaturated fatty acids or coenzyme A derivatives, for these have been shown to be ineffective as substrates or stimulators for the reaction.

Recent reports from several laboratories have demonstrated a phospholipid requirement for enzymes associated with mitochondrial electron transport after treatment of particles with cholate or deoxycholate. Several phospholipids were shown to stimulate cytochrome oxidase activity (35, 36). A rather specific requirement for phosphatidylethanolamine containing unsaturated fatty acids for D(-)-α-hydroxybutyrate dehydrogenase activity has been reported (37). In the latter case it was pointed out that approximately 100 to 400 molecules of phosphatidylethanolamine combined with each molecule of enzyme, and it was concluded that "this high molar ratio argues against a simple coenzyme function for lecithin."

The possibility that exogenous lipids serve only to activate the cyclopropane acid-synthesizing system of C. butyricum extracts is unattractive for several reasons. The product of the enzymatic reaction is clearly phosphatidylethanolamine containing cyclopropane fatty acids. The most stimulatory exogenous lipid mixture (S. marcescens "unsaturated" lipids) is very rich in phosphatidylethanolamine which contains unsaturated fatty acids. A similar dispersion of bacterial lipids containing a high proportion of phosphatidylethanolamine which is devoid of unsaturated fatty acids (S. marcescens "saturated" lipid) is a very poor substrate. Finally, the previously discussed discrepancy between the available substrate in the endogenous lipid and the observed amount of product formed is not in accord with a nonsubstrate function for the exogenous lipid. It must also be emphasized that phospholipid activation of cytochrome oxidase and D(-)-α-hydroxybutyrate dehydrogenase is demonstrable only after treatment with detergent, which results in removal of normally bound phospholipid.

A highly purified, homogeneous, bacterial phosphatidylethanolamine prepared by us has thus far resisted all attempts to produce a suitable aqueous dispersion. Lipids not forming aqueous micellar solutions are inactive in the lipid-stimulated C. butyricum reaction. For this reason, some uncertainty regarding the specificity for lipid substrate remains. However, the most parsimonious hypothesis which can be advanced in the light of available information is that cyclopropane fatty acid synthesis requires S-adenosylmethionine and a phospholipid, probably phosphatidylethanolamine, as substrates. If this is true, this reaction represents the first instance of an enzymatic transformation of fatty acid esterified in phospholipid. The questions of intermediates, the number of enzymatic steps in the overall conversion, and the exact stoichiometry of the reaction cannot be answered at present. The product of the reaction observed in crude extracts is phosphatidylethanolamine containing cyclopropane acids.

**SUMMARY**

1. Extracts of either Serratia marcescens or Clostridium butyricum are capable of forming labeled cyclopropane fatty acids when they are incubated with S-adenosylmethionine labeled in the methyl group. Methionine itself is not an effective precursor; neither are other 1 carbon donors such as formate, formaldehyde, and serine.

2. The enzyme system of Serratia marcescens contains endogenous lipid substrates for this reaction which cannot be removed or replaced by simple manipulations. Various unsaturated fatty acid derivatives fail to enhance the rate of the reaction. Operations which are ordinarily employed to remove small cofactor molecules from enzymes do not diminish the rate of reaction catalyzed by these extracts.

3. In contrast, the enzyme system of Clostridium butyricum fails to catalyze the reaction unless aqueous dispersions of phospholipids with unsaturated fatty acids in glycerol ester linkage are added. The exact nature of the lipid substrates in the reaction observed has not been defined with certainty, but the effective dispersions contain phosphatidylethanolamine as the principal component.

4. The product of the reaction, isolated from incubation mixtures of either enzyme source, is phosphatidylethanolamine which contains labeled cyclopropane fatty acids.

**REFERENCES**

Enzymatic Synthesis of Cyclopropane Fatty Acids Catalyzed by Bacterial Extracts
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