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 Daehky and As- selineau (6) and characterized as cis-9,10-methylenecadecanoic 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 36° 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 inoculation (13). Cells were harvested in the log phase of growth by centrifugation and washed once with water or 1% 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 X g for 30 minutes and discarded. The supernatant solution obtained by centrifugation at 100,000 X g for 1½ hours was adjusted to neutrality with 1 M 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. (10), 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 acid esters were assayed by gas-liquid chromatography generated from N-methylnitrosourea. Gas-liquid chromatographic analysis of fatty acid esters of 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 Mallinkrodt 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 H$_2$SO$_4$ (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 xerox" x ray film between the chromatogram and a matching glass plate.

**Materials**

*S. marcescens* "Unsaturated" Lipids—Total lipids were extracted by the method of Folch, Lees, and Sloane Stanley (21) 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 either by sonication of suspensions in 0.1 m phosphate buffer, pH 7.0, 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, \( R_F 0.53 \)) corresponding to phosphatidyl-ethanolamine, and two weaker, ninhydrin-negative spots (\( R_F 0.79 \) 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,\(^a\) 45%; 16:1, 17%; 17:CY, 23%; 18:1, 11%; 19:CY, 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; phosphatidyl-ethanolamine was the major constituent. The fatty acid composition was: 16:0, 49%; 17:CY, 41%; 19:CY, 9%.

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

**Synthetic and Commercial Lipids**—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 phosphatidylethanolamine 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 \(-20^\circ\) at pH 5. Under these conditions, the compound is stable for many months. S-Adenosyl-l-homocysteine was made by the procedure of Sakami (28). All radioactive compounds used in these experiments were purchased from New England Nuclear Corporation, 675 Albany Street, Boston 16, Massachusetts.

**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-adenosylmethionine 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 those 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-C$^14$-serine.\(^4\) 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.

**Nature of Lipid Acceptor for Cyclopropane Fatty Acid Synthesis**—In the experiments of Table I and II it should be noted that crude extracts of *S. marcescens* were used and that no other source of unsaturated fatty acid was added. These crude extracts contained a fairly high level of endogenous lipid (~80 \( \mu \)g per mg of protein), sufficient, in fact, to make unnecessary the addition of carrier fatty acids before gas-liquid chromatography of the radioactive fatty acid products. Attempts to remove endogenous lipid in order to examine the effect of added potential lipid substrate were completely unsuccessful in the case of *S. marcescens* extracts. Dialysis or treatment with Sephadex G-50 (Pharmacia, Uppsala, Sweden) removed some

\(^a\) The abbreviations used for fatty acids are as follows: 16:0, hexadecanoic acid; 16:1, hexadecenoic acid; 17:CY, methylene hexadecanoic acid (cyclopropane); etc.

\(^4\) S. Pohl, and J. H. Law, unpublished observations.
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 μM; phosphate, pH 7.0, 15 to 20 μMol; extract, 1 mg of protein. Reaction time, 6 minutes at 37°C. In Experiment 2, each reaction contained, in 0.5-ml volume: uniformly labeled threonine-CH₃, 15 μMoles (182,000 d.p.m.), or AMe-CH₃-H₃; 8 μMoles (170,000 d.p.m.); extract, 2.5 mg of protein; buffer and NaCN, similar to Experiment 1. Reaction time, 10 minutes at 37°C. Experiment 3: AMe-CH₃-H₃ or L-methionine-CH₃-H₃, 5 pmol (129,000 d.p.m.); extract, 1 mg of protein; buffer and NaCN, similar to Experiment 1. Reaction time, 10 minutes at 37°C. 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>AMe-CH₃-C₄</td>
<td>580</td>
</tr>
<tr>
<td>Serine-3-C¹⁴</td>
<td>112</td>
</tr>
<tr>
<td>Formate-C¹⁴</td>
<td>0</td>
</tr>
<tr>
<td>Formaldehyde-C¹⁴</td>
<td>0</td>
</tr>
<tr>
<td>AMe-CH₃-H₃</td>
<td>2840</td>
</tr>
<tr>
<td>Uniformly labeled serine-C¹⁴</td>
<td>0</td>
</tr>
<tr>
<td>AMe-CH₃-H₃</td>
<td>2460</td>
</tr>
<tr>
<td>Methionine-CH₃-H₃</td>
<td>41</td>
</tr>
</tbody>
</table>

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

### Table I

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

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0-16:1</td>
<td>1.9</td>
</tr>
<tr>
<td>17:Cy</td>
<td>82</td>
</tr>
<tr>
<td>18:0-18:1</td>
<td>6.9</td>
</tr>
<tr>
<td>19:Cy</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, 16:1, 17:Cy, 18:0, 18:1, 19:Cy.† Recovery of radioactivity from the column was 76%.

### Table II

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

<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:Cy</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:Cy</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, 16:1, 17:Cy, 18:0, 18:1, 19:Cy.† Recovery of radioactivity from the column was 76%.

### Table III

**Lipid substrate requirement for cyclopropane fatty acid synthesis catalyzed by soluble extracts of *C. butyricum***

<table>
<thead>
<tr>
<th>Addition</th>
<th>H³ incorporated into fatty acids (d.p.m.)</th>
<th>Cyclopropane acids formed (μmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>S. marcescens</em> “unsaturated” lipid, 3 mg</td>
<td>3690</td>
<td>9</td>
</tr>
<tr>
<td><em>S. marcescens</em> “saturated” lipid, 3 mg</td>
<td>253</td>
<td>7</td>
</tr>
<tr>
<td>Palmitoleyl-CoA, 1.0 μmol</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Palmitoleic acid, 4.5 μmol</td>
<td>29</td>
<td>1</td>
</tr>
</tbody>
</table>

* This material failed to give a suitable aqueous dispersion, in accord with the observations of Fleisher and Klouwen (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 \textit{S. marcescens} extract and by \textit{C. butyricum} extract. In each case, radioactivity incorporated into cyclopropane acids is chromatographically coincident with lipid phosphorus. Material contained in a first peak from the reaction catalyzed by the \textit{S. marcescens} extract did not contain radioactive fatty acids and was not investigated further.

Further identification of the reaction product from the experiment with \textit{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 \textit{C. butyricum} extract.

![Fig. 1](https://example.com/fig1.png)

**Fig. 1.** Silicic acid chromatography of enzymatic reaction product obtained with \textit{S. marcescens} extract. A reaction mixture containing AMe-CH$_3$-C$^{14}$, 20 mmoles (100,000 d.p.m.); NaCN, 10 amoles; \textit{S. marcescens} extract, 4.5 mg of protein; and phosphate buffer, pH 7.0, 20 mmoles, in a final volume of 1.0 ml was incubated for 20 minutes at 37°C. A second addition of AMe-CH$_3$-C$^{14}$ (100,000 d.p.m.) was made and the reaction was run for an additional 20 minutes. Carrier \textit{S. marcescens} lipid (70 mg) was added, and the reaction mixture was immediately extracted three times with 10 ml of CHCl$_3$-CH$_2$OH (2:1). The combined extract was washed three times with 8 ml of H$_2$O. The washings were evaporated to dryness, the residue was taken up in 5 ml of CHCl$_3$, and this solution was applied to a column of silicic acid, 1 X 16 cm. A CHCl$_3$-CH$_2$OH gradient (200 ml of CHCl$_3$ in mixing chamber and 200 ml of CH$_2$OH in reservoir) was used for elution, and 5-ml fractions were collected automatically. The symbols are: \(\bullet\)---\(\bullet\), lipid phosphorus; \(\bigcirc\)--\(\bigcirc\), C$^{14}$.

![Fig. 2](https://example.com/fig2.png)

**Fig. 2.** Silicic acid chromatography of enzymatic reaction product obtained with \textit{C. butyricum} extract. A reaction mixture containing AMe-CH$_3$-C$^{14}$, 1.5 mmoles (2 X 10$^4$ d.p.m.); \textit{S. marcescens} "unsaturated" lipid, 13.5 mmoles of lipid phosphorus; dialyzed \textit{C. butyricum} extract, 36 mg of protein; and Tris, pH 7.0, 47 mmoles, in a volume of 3.0 ml was incubated for 44 hours at 30°C. The product was extracted and washed as described in Fig. 1. The extracted lipid contained 1.7 X 10$^4$ d.p.m. (86% yield). An aliquot was applied to a column of silicic acid, 0.5 X 18 cm, which was eluted with a CHCl$_3$-CH$_2$OH gradient similar to that described in Fig. 1. An aliquot of the eluent was assayed for lipid phosphorus (\(\bullet\)---\(\bullet\)) and counted for C$^{14}$ (\(\bigcirc\)--\(\bigcirc\)).

![Fig. 3](https://example.com/fig3.png)

**Fig. 3.** Thin layer chromatography of the CHCl$_3$-CH$_2$OH-extracted reaction product before silicic acid chromatography. Lipid samples were spotted on the plate, which was then developed in CHCl$_3$-MeOH-H$_2$O (65:25:4). After radioautography as described in the text, the spots were visualized by exposure to iodine vapors. A tracing of the developed chromatogram is shown superimposed over the radioautograph. The columns are as follows: 1, palmitoleic acid; 2, synthetic dipalmityolphosphatidylethanolamine; 3, reaction product. Faintly visible spots, 0.5 and 2 cm below the intense spot on the radioautograph, failed to be reproduced in the photograph.
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>Concentration</th>
<th>Radioactivity recovered*</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0-16:1</td>
<td>0</td>
<td>0 %</td>
</tr>
<tr>
<td>17:0</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>19:0</td>
<td>12,700</td>
<td>66.1 %</td>
</tr>
<tr>
<td>&gt;19:0</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 phosphotidylethanolamine (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-hydroxymercruribenzoate inhibition which is reversed

<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>None</td>
<td>d.p.m.</td>
</tr>
<tr>
<td>p-Hydroxymercruribenzoate (10-3 M)</td>
<td>4900</td>
</tr>
<tr>
<td>p-Hydroxymercruribenzoate (10-3 M) + cysteine (2 X 10-3 M)</td>
<td>580</td>
</tr>
<tr>
<td>Iodosuccinate (10-3 M)</td>
<td>4380</td>
</tr>
<tr>
<td>Mercaptoethanol (10-3 M)</td>
<td>6000</td>
</tr>
<tr>
<td>Adenosylhomocysteine (4 X 10-4 M)</td>
<td>5600</td>
</tr>
<tr>
<td>Cysteine (2 X 10-4 M)</td>
<td>5140</td>
</tr>
</tbody>
</table>
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 cul-
tures. The origin of the methylene bridge from 1 carbon dono-
(reserve 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 cyclo-
propane acids, and reincorporated into phospholipids, or the
synthesis of cyclopropane acids must require intact phospho-
lipid as substrate (10). This is a particularly important point
in the case of gram-negative bacteria like Escherichia coli (33)
monitored in the case of gram-negative bacteria like Escherichia coli (33)
ununsaturated fatty acids are completely converted to cyclopropane
acids. In contrast, fatty acids or coenzyme A derivatives, for these have been shown to
be ineffective as substrates or stimulators for the reaction.

Cyclopropane fatty acids of C. butyricum extracts, but stimulatory to
the S. marcescens reaction.

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 a

effective precursor; neither are other 1 carbon donors such as formate,
formaldehyde, and serine.

2. The enzyme system of Serratia marcescens contains en-
dogenous lipid substrates for this reaction which cannot be
removed or replaced by simple manipulations. Various un-
saturated 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.

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