Biosynthesis of Cyclopropane Compounds

IX. STRUCTURAL AND STEREOCHEMICAL REQUIREMENTS FOR THE CYCLOPROpane SYNTHETASE SUBSTRATE*

(Received for publication, May 31, 1966)

PAUL J. THOMAS† AND JOHN H. LAWS‡

From the James Bryant Conant Laboratory, Harvard University, Cambridge, Massachusetts 02138

SUMMARY

Several natural and synthetic phospholipids can serve as substrates for the cyclopropane synthetase enzyme of Clostridium butyricum. The conditions for optimum substrate effectiveness vary with the substrate; e.g. an anionic surface-active agent is necessary with phosphatidylethanolamine, while divalent cations stimulate the rate of reaction with anionic phospholipids. These variable conditions probably provide the optimal surface charge on the phospholipid aggregates for most efficient enzyme-substrate interaction.

A diether analogue of phosphatidylethanolamine, 1,2-di-(9-octadecenlyoxy)-3-(2-aminoethy1phosphorylpropane, is an effective substrate for cyclopropane synthetase, indicating that the ester carbonyl groups are not essential for activity.

When presented with a racemic mixture of dioleyl phosphatidylethanolamines, the enzyme acts nearly exclusively upon the 3-phosphoglycerol isomer.

Cyclopropane synthetase acts upon phospholipids deposited upon filter paper discs. The pattern of activity with various phospholipids is different from that with the compounds dispersed in buffer, and no accessory substances such as surface-active agents or divalent cations stimulate the reaction on filter paper.

We have previously described the properties of an enzyme which carries out Reaction 1 (1-4). The most effective phospholipid substrate that we have found for this enzyme is a suitably dispersed preparation of phosphatidylethanolamine contaminated with an anionic surface-active agent (2). The role of the surface-active agent may be to impart to the multimolecular substrate aggregation a surface charge complementary to that of the enzyme, as proposed by Bangham and Dawson (5).

The studies of van Deenen and de Haas (6) on the specificity and mechanism of phospholipase A, which carries out Reaction 2, have shown that the enzyme has little specificity for the nature of R₁, R₂, or R₃, but that it is absolutely specific for the stereochemistry about the center glycerol carbon, attacking only the natural 3-phosphoglycerol derivatives.

We have examined some structural and stereochemical features of phospholipid molecules which act as substrates for cyclopropane synthetase. The results reported here indicate that cyclopropane synthetase shows a specificity pattern similar to that of phospholipase A in its essentially exclusive requirement for a 3-phosphoglycerol derivative and a general lack of specificity for the nature of the base or of the alkyl chains. However, as shown earlier (3) and confirmed here, cyclopropane synthetase, unlike phospholipase A, has only a slight preference for the position of an alkyl chain in the phospholipid molecule.

EXPERIMENTAL PROCEDURE

Materials—Soybean phosphatidylethanolamine and bovine brain phosphatidylserine were purchased from Applied Science Laboratories. Phosphatidylethanolamine was also isolated from the lipids of Azotobacter agilis, ATCC 9104, by chromatography on DEAE-cellulose as described previously (2). From the same lipid mixture, it was possible to isolate phosphatidylglycerol by elution of the DEAE column with a solution of chloroform-methanol, 4:1 (v/v), which contained 2% v/v concentrated aqueous ammonia and 1.9% w/v ammonium acetate. The crude phosphatidylglycerol thus obtained was purified by

---

* This research was supported by Grant GB 3170 from the National Science Foundation, and in part by Career Program Award 8521 from the United States Public Health Service.
† Predoctoral fellow of the United States Public Health Service. Present address, Department of Pharmacology, University of Wisconsin, Madison, Wisconsin 53706.
‡ Present address, Department of Biochemistry, University of Chicago, Chicago, Illinois 60637.
§ Present address, Department of Pharmacology, University of Wisconsin, Madison, Wisconsin 53706.

1 This enzyme was previously called cyclopropane fatty acid synthetase. In light of the experiments reported here, which show that compounds which do not contain fatty acids can be substrates for this enzyme, the name cyclopropane synthetase is now used.

2 Reaction 1:

Unsaturated phospholipid + S-adenosyl-L-methionine →

cyclopropane phospholipid + S-adenosyl-L-homocysteine (1)

3 Reaction 2:

R₁COCH₂OH → HOCH₂COCH₂R₃ (2)


Phosphatidic acid was prepared by phosphorylation of a 1,2-diglyceride by the method of Baer and Buchnea (9). The diglyceride was obtained from olive oil by treating with pancreatin, Bailey, and Kates (10). The combined products from olive oil were applied to a column of silicic acid, which was developed with hexane and eluted with a gradient of ether in hexane (11). Fractions were monitored by thin layer chromatography. A diglyceride fraction (650 mg) was largely the 1,2-isomer, but contained a small amount of the 1,3-compound as well. Phosphorylation of this diglyceride afforded an 80% yield of product when it was carried out under strictly anhydrous conditions. The monosodium salt of phosphatidic acid, purified by acetone precipitation and silicic acid chromatography, gave only one spot on a thin layer chromatogram, and contained 4.2% phosphorus. Deacylation followed by paper chromatography (12) gave only one spot corresponding to α-phosphoglycerol.

Phosphatidic acid was then converted to phosphatidylglycerol by the reaction of phosphatidic acid with a natural mixture of phospholipids, which contained mostly phosphatidylethanolamine admixed with phosphatidylglycerol and traces of other lipids. This mixture was found to be a more effective substrate for cyclopropane synthesis than a purified phosphatidylethanolamine (1). However, pure phosphatidylethanolamine can be rendered even more effective than the crude natural mixture by contamination with a variety of anionic surface-active agents. It seemed likely that phosphatidylglycerol was serving as a natural surface-active agent in the crude mixtures. When this idea was tested, it was found that phosphatidylglycerol is in fact a substrate, but that it can also enhance the rate of conversion of exogenous phosphatidylethanolamine to cyclopropane phospholipid (Table I). However, in the presence of sodium dodecyl sulfate and in the absence of divalent cations, the rate of conversion of phosphatidyglycerol is suppressed, while that for phosphatidylethanolamine is enhanced. For the conversion of phosphatidylglycerol, cationic and nonionic detergents had little effect, while the addition of the divalent cations (calcium or magnesium) produced an approximate doubling of the rate. Therefore, the conditions under which a potential substrate is tested must be varied in order to find those most effective for its enzymatic conversion.

Earlier results (1) had indicated that various phospholipids could serve as substrates for the cyclopropane synthetase reaction, but all were less effective than phosphatidylethanolamine. The substrate specificity of the enzyme was then re-examined with very pure preparations of natural and synthetic phospholipids under a variety of conditions. The labeled products in each case were extracted at the end of the reaction and chromatographed on thin layers of silicic acid. Radioautography was used to compare Rf values with standards. The initial velocities for each substrate under the best conditions are given in Table II.

In most cases, these values were determined at the highest lipid concentrations obtainable (1 to 2 mM), except for phosphatidylserine, which was found to give lower rates as the lipid concentrations were increased beyond 1 mM. In the case of the two-phase assay, between 80 and 90% of the radioactive material could be extracted from the discs, while more than 95% of the radioactive material was extracted from the discs used in the Goldfine assay method (16) under similar conditions.
Effectiveness of phosphatidylglycerol as substrate and as anionic surface-active agent in cyclopropane synthetase reaction

Incubation mixture contained phosphatidylethanolamine, 0.95 \mu \text{mole}; 
Tri-acetate (pH 7), 60 \text{ mmoles}; S-adenosylmethionine-methyl-\textsuperscript{14}C (700 \text{ cpm per \text{mumole}}), 80 \text{ mmoles}; and crude dialyzed enzyme, 2 \text{ \mu g}. Total volume was 1.0 \text{ ml} and samples were incubated for 30 min at 30\degree. Product was determined by the extraction assay procedure. Some phospholipid is always present in crude enzyme preparations and gives a considerable endogenous rate. Products were extracted with chloroform-methanol (2:1). Lipids were chromatographed on thin layer plates and the appropriate areas were scraped to loosen the silicic acid. Lipids were eluted from the collected silicic acid with acid. Lipids were eluted from the collected silicic acid with.

Concentration was increased. The value given was, therefore, determined at a low lipid concentration (0.25 \text{ mM}).

Enzymatic Cyclopropane Synthesis with Phospholipids Incorporated on Filter Paper—We have stressed the importance of proper dispersion of phospholipid substrates for obtaining maximum enzymatic reaction rates (1). In our experience, dispersion by the technique of Fleischer and Klouwen (17) has been most successful. We were, therefore, surprised to discover that phospholipids simply deposited onto pieces of filter paper and presented to an enzymatic reaction mixture could serve as substrates, although less effectively than dispersed phospholipids. These experiments were attempted because of the successful application of the Goldfine filter disc assay (16), in which aliquots taken from enzymatic reaction mixtures are transferred to filter paper and the phospholipids are precipitated in the fibers by application of the Goldfine filter disc assay (16), in which aliquots taken from enzymatic reaction mixtures are transferred to filter paper and the phospholipids are precipitated in the fibers by.

<table>
<thead>
<tr>
<th>Substrate added</th>
<th>Amount</th>
<th>Products containing cyclopropane acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\mu \text{mole}</td>
<td>Phosphatidylethanolamine</td>
</tr>
<tr>
<td>1. Phosphatidylethanolamine</td>
<td>1.9</td>
<td>3534</td>
</tr>
<tr>
<td>2. Phosphatidylglycerol</td>
<td>1.0</td>
<td>1222</td>
</tr>
<tr>
<td>3. Phosphatidylethanolamine + phosophatidylglycerol</td>
<td>1.9</td>
<td>3444</td>
</tr>
<tr>
<td>4. Phosphatidylethanolamine + phosophatidylglycerol + sodium dodecyl sulfate</td>
<td>1.0</td>
<td>3888</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

Further characterization of these products is in progress.

Other conditions of incubation were as in Table I, except that enzyme purified through the DEAE step (2) was used. All assays were by filter disc assay of Goldfine (7).

Table II

<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
<th>Conditions of incubation</th>
<th>Initial rate</th>
<th>\text{pmoles/hr/mg protein}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidylethanolamine</td>
<td>A. agilise</td>
<td>SDS, 5 x 10\textsuperscript{-4} M</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>Soybean\textsuperscript{c}</td>
<td>SDS, 5 x 10\textsuperscript{-4} M</td>
<td>Nil\textsuperscript{e}</td>
<td></td>
</tr>
<tr>
<td>Phosphatidylglycerol</td>
<td>A. agilise\textsuperscript{b}</td>
<td>Ca\textsuperscript{+}, 10\textsuperscript{-4} M</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Phosphatidic acid</td>
<td>Synthetic</td>
<td>Ca\textsuperscript{+}, 10\textsuperscript{-4} M</td>
<td>6\textsuperscript{f}</td>
<td></td>
</tr>
<tr>
<td>Phosphatidylserine</td>
<td>Bovine brain</td>
<td>No additions</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Fatty acid composition as reported by Hildebrand and Law (3).

\textsuperscript{b} SDS, sodium dodecyl sulfate.

\textsuperscript{c} Applied Science Laboratories, composition as determined by gas-liquid chromatography: 16:0, 25\%; 18:0, 7\%; 18:1, 1\%; 18:2, 60\%; 18:3, 7\%.

\textsuperscript{d} The low rate seems to result from the small amount of mono-unsaturated acid and the fact that polyunsaturated acid residues react very only slowly. Examination of the product obtained with adenosymethionine of very high specific activity showed that radioactive carbon was incorporated in small amounts into fatty acids which had the chromatographic properties of a monounsaturated cyclopropane acid and a diolefinic cyclopropane acid.

\textsuperscript{e} Fatty acid composition is virtually identical with that of phosphatidylethanolamine from the same organism (3).

\textsuperscript{f} The product showed three radioactive spots on a thin layer plate, one of which corresponded to the starting material. It is not clear whether this is the result of degradation during reaction or isolation or whether it is due to different salt forms of phosphatidic acid.

\textsuperscript{g} Applied Science Laboratories, composition as determined by gas-liquid chromatography: 16:0, 3\%; 18:0, 42\%; 18:1, 37\%; 18:2, 2\%; later than 18:2, unidentified, 16\%.
FIG. 1. Radioactivity incorporated into phosphatidyglycerol with respect to time in the two-phase assay. Curve A, 0.1 μmole of lipid per disc; Curve B, 0.05 μmole of lipid per disc; Curve C, no added lipid; Curve D, no enzyme. Each disc was incubated in a total volume of 0.25 ml containing 25 μmole of 2-phospholylglycerol-methyl-14C (2 mCi per mmole), 15 μmole of Tris-acetate buffer (pH 7), and 0.02 mg of DEAE enzyme, at 30° for 0, 10, 15, or 20 min as shown.

TABLE III
Lipids impregnated in filter paper as substrates for cyclopropane synthetase.

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Initial rate (μg/mg protein/hr/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidylethanolamine (A. agilis)</td>
<td>5</td>
</tr>
<tr>
<td>Phosphatidylglycerol (A. agilis)</td>
<td>11</td>
</tr>
<tr>
<td>Phosphatidic acid</td>
<td>3</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
<td>1</td>
</tr>
<tr>
<td>Neutral lipids</td>
<td>1</td>
</tr>
<tr>
<td>Control, no lipid</td>
<td>1</td>
</tr>
</tbody>
</table>

* A mixture of fatty acids and mono-, di-, and triglycerides produced by the action of pancreatic lipase on olive oil.

For exploring this idea, a diether phosphatidylethanolamine, 1,2-di(9-octadecenoyloxy)-3-(2-aminoethyl)phosphorylpropane was prepared (15) and tested as a substrate. When dispersed in aqueous buffer, it gave an initial rate of 7 μmole per hour per mg of protein when incubated with sodium dodecyl sulfate under the conditions described in Table II. It was also tested in the two-phase assay on filter paper, where it gave an initial rate of 3 μmole per hour per mg of protein under the conditions described in Table III. It is important to remember that the diether analogue was a racemic mixture, and it is likely, from the results discussed below, that only one isomer reacted with the enzyme.

The enzymatic product from incubation of the diether analogue was chromatographed on a thin layer of silica gel; the 14C-labeled product had the same Rf value as the starting material. The chromatographic properties of the labeled product were unchanged after treatment with 0.05 N NaOH in 50% ethanol at 37° for 20 min, while a control sample of diacyl phosphatidylethanolamine was completely destroyed under the same conditions.

The results indicate that the diether compound is a moderately good substrate for the enzymatic reaction, and, therefore, the intact phospholipid structure undergoes reaction in the course of cyclopropane ring synthesis. The ester groups are not essential for the reaction. These results verify and amplify the findings of Chung and Goldfine (20), who reported that vinyl ether chains could serve as substrates for cyclopropane synthetase. It would seem that the earlier trivial name given to this enzyme, cyclopropane fatty acid synthetase, is too specific, and hence the more general name, cyclopropane synthetase, has been used here.

Cyclopropane Synthesis with Racemic Substrate—van Deenen and de Haas (6), in their definitive study of the specificity of phospholipase A, showed that the enzyme hydrolyzes the 2-acyl group only from phospholipids derived from β-phosphoglycerol. These experiments raised a question of the steric specificity of the cyclopropane synthetase and suggested an experimental approach to the problem. A racemic phosphatidylethanolamine was prepared (13) and reacted with cyclopropane synthetase and S-adenosylmethionine-methyl-14C, and the products were analyzed with phospholipase A.

A solution of synthetic dioleoyl phosphatidylethanolamine (approximately 10 mg) in 0.2 ml of benzene was applied to a 5 cm circle of Whatman No. 1 filter paper. The benzene was allowed to evaporate, and the paper was placed in the bottom of a 250-ml Erlenmeyer flask. A solution containing 0.3 ml of 1 m Tris-acetate buffer at pH 7, 0.3 ml of DEAE-purified enzyme solution (2.2 mg per ml) and 0.5 ml of S-adenosylmethionine-methyl-14C (0.3 μmole, 10 μC), and 3.9 ml of water was added. The flask was incubated with shaking for 60 min at 30°, and then an additional 0.3 ml of enzyme and 10 μC of S-adenosylmethionine were added, and incubation was continued for another 60 min. The reaction was terminated by the addition of 50 ml of cold 10% trichloracetic acid, and the disc was washed.

TABLE IV
Analysis of products from action of cyclopropane synthetase on racemic phosphatidylethanolamine

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Phosphatidylethanolamine</th>
<th>Lysophosphatidylethanolamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aliquot (20%) of labeled product before phospholipase treatment</td>
<td>24,300 cpm</td>
<td>22,500 cpm</td>
</tr>
<tr>
<td>Aliquot (80%) of labeled product after phospholipase treatment</td>
<td>24,300 cpm</td>
<td>3,400 cpm</td>
</tr>
<tr>
<td>Phosphorus content of phospholipase products</td>
<td>116 μg</td>
<td>74,700 cpm</td>
</tr>
</tbody>
</table>

The results indicate that the diether compound is a moderately good substrate for the enzymatic reaction, and, therefore, the intact phospholipid structure undergoes reaction in the course of cyclopropane ring synthesis. The ester groups are not essential for the reaction. These results verify and amplify the findings of Chung and Goldfine (20), who reported that vinyl ether chains could serve as substrates for cyclopropane synthetase. It would seem that the earlier trivial name given to this enzyme, cyclopropane fatty acid synthetase, is too specific, and hence the more general name, cyclopropane synthetase, has been used here.

Cyclopropane Synthesis with Racemic Substrate—van Deenen and de Haas (6), in their definitive study of the specificity of phospholipase A, showed that the enzyme hydrolyzes the 2-acyl group only from phospholipids derived from β-phosphoglycerol. These experiments raised a question of the steric specificity of the cyclopropane synthetase and suggested an experimental approach to the problem. A racemic phosphatidylethanolamine was prepared (13) and reacted with cyclopropane synthetase and S-adenosylmethionine-methyl-14C, and the products were analyzed with phospholipase A.

A solution of synthetic dioleoyl phosphatidylethanolamine (approximately 10 mg) in 0.2 ml of benzene was applied to a 5 cm circle of Whatman No. 1 filter paper. The benzene was allowed to evaporate, and the paper was placed in the bottom of a 250-ml Erlenmeyer flask. A solution containing 0.3 ml of 1 m Tris-acetate buffer at pH 7, 0.3 ml of DEAE-purified enzyme solution (2.2 mg per ml) and 0.5 ml of S-adenosylmethionine-methyl-14C (0.3 μmole, 10 μC), and 3.9 ml of water was added. The flask was incubated with shaking for 60 min at 30°, and then an additional 0.3 ml of enzyme and 10 μC of S-adenosylmethionine were added, and incubation was continued for another 60 min. The reaction was terminated by the addition of 50 ml of cold 10% trichloracetic acid, and the disc was washed.

TABLE IV
Analysis of products from action of cyclopropane synthetase on racemic phosphatidylethanolamine

An aliquot of labeled phospholipid produced by the action of cyclopropane synthetase on racemic phosphatidylethanolamine and S-adenosylmethionine-methyl-14C was applied to one-half of a thin layer plate. A second aliquot was treated with phospholipase A and the phospholipase products were applied to the other half of the thin layer plate. After chromatography, the products were located, eluted from the silicic acid, and assayed as described in the text.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Phosphatidylethanolamine</th>
<th>Lysophosphatidylethanolamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aliquot (20%) of labeled product before phospholipase treatment</td>
<td>24,300 cpm</td>
<td>22,500 cpm</td>
</tr>
<tr>
<td>Aliquot (80%) of labeled product after phospholipase treatment</td>
<td>24,300 cpm</td>
<td>3,400 cpm</td>
</tr>
<tr>
<td>Phosphorus content of phospholipase products</td>
<td>116 μg</td>
<td>74,700 cpm</td>
</tr>
</tbody>
</table>
as described for the two phase assay. After the final water wash, the disc was extracted with 50 ml of 95% ethanol and then twice with 50-ml portions of chloroform-methanol (2:1). The combined extracts were taken to dryness.

An aliquot of the lipid product was chromatographed on a thin layer of silicic acid. The remainder was treated with phospholipase A to the limit of hydrolysis as described by Hildebrand and Law (9), and the phospholipase products were also chromatographed on a thin layer of silicic acid. All products were located by staining with iodine and comparing with authentic standards. The radioactive products were scraped from the plates with silicic acid and eluted with acidic methanol in ether. The eluted lipids were assayed for radioactivity and for phosphorus. The results are presented in Table IV. These show that the phospholipase reaction had proceeded nearly to completion, for the phosphorus content of the unhydrolyzed 1-isomer of phosphatidylethanolamine is nearly equal to that of the 3-phosphoglyceride lysophosphatidylethanolamine. Nearly all of the labeled fatty acids were, therefore, associated with the 3-phosphoglyceride. In fact, the small amount of radioactivity remaining in phosphatidylethanolamine may represent unhydrolyzed 3-phosphoglyceride. The distribution of radioactivity in free fatty acid, derived from the secondary alcohol ester, and in the lyso compound, representing fatty acid esterified to the primary hydroxyl, is in excellent accord with the positional preference determined earlier for cyclopropane synthetase (3). Clearly, the enzyme has a high degree of specificity for the diacyl 3-glycerophosphorylethanolamine isomer.

**DISCUSSION**

As we have shown earlier (2), the rate of the cyclopropane synthetase reaction is stimulated by anionic surface-active agents and inhibited by cationic surface-active agents. This has been interpreted as the result of penetration of the charged surface-active agent into the phospholipid bilayer to give a charge on the lipid aggregate which is more or less complementary to the charge on the protein surface. Ideally, matching of charges would then permit enzyme-lipid contact at the maximum rate. The interpretation is based upon the work of Bangham and Dawson (5). The role of calcium ions in facilitating the reaction of anionic phospholipids (Table II) is also interpreted as a surface charge phenomenon. The behavior of phosphatidylserine, which gives a decreasing rate of enzymatic reaction as the lipid concentration is increased, is reminiscent of the effect of this lipid in blood clotting (21), during which it has been observed that certain concentrations are inhibitory. This may reflect a change in the organization of lipid molecules, and hence the surface charge, with concentration.

Some idea of the nature of the surface which the dispersed phospholipid substance presents to the enzyme can be gained by examination of electron micrographs of Fleischer-Klouwen dispersions of phosphatidylethanolamine made by Rothfield and Horne (8). These showed sheets of bimolecular leaflets folded and rolled together resembling those given by Bangham (23). The first step in the enzymatic process must, therefore, be the approach of the enzyme to the charged wall of phospholipid, in which matching of charges is an important factor.

In a second step, a phospholipid molecule must be bound to the enzyme, and the stereochemistry of the glycerol backbone must play a dominant role here. This is indicated by the relative lack of specificity for the nature of the second alcohol of the phosphodiester and the nature of the alkyl linkages to the glycerol, and the nearly absolute specificity for reaction with the 3-phosphoglycerol compound. The alkyl chain on the glycerol primary hydroxyl group occupies the same orientation in either the 1- or 3-phosphoglycerol compound. The fact that the chain is only acted upon in the 3-isomer indicates that in the other isomer it never reaches the active site. This can best be explained in terms of a binding site into which only the 3-isomer can fit.

Finally, there is the alkylation step, about which we know very little. How and when the second substrate, S-adenosylmethionine, enters into the reaction is still unclear.

If there is merit to the suggestion that the cyclopropane synthetase enzyme recognizes a vast wall of phospholipid in a bimolecular leaflet, then it is reasonable that in the cell it may recognize a similar wall of leaflet attached to a protein matrix, the cell membrane. The observation that phospholipids impregnated into filter paper can serve as substrates for the enzyme then takes on added interest. Is the cellular surface serving the function similar to that provided by the protein surface of the cell membrane? Will different enzymatic rates be observed if different matrix materials are used? These questions provide material for future investigations which may be useful in probing the nature of the cell membrane.

Acknowledgments—Dr. Albert Chung performed some of the preliminary experiments with phosphatidylglycerol which led to the work reported here. He also read the manuscript and offered valuable suggestions. We wish to thank Miss Birgitta Essén for excellent technical assistance.

**REFERENCES**


Biosynthesis of Cyclopropane Compounds: IX. STRUCTURAL AND STEREOCHEMICAL REQUIREMENTS FOR THE CYCLOPROPANE SYNTHETASE SUBSTRATE
Paul J. Thomas and John H. Law


Access the most updated version of this article at http://www.jbc.org/content/241/21/5013

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/241/21/5013.full.html#ref-list-1