The Enzymatic Acylation of Lysophosphatidylinositol*

R. W. KEENAN† AND LOWELL E. HOKIN‡

From the Department of Physiological Chemistry, University of Wisconsin, Madison 6, Wisconsin

(Received for publication, December 26, 1963)

Lysophosphatidylcholine (1), lysophosphatidylethanolamine (2), and lysophosphatidic acid (3) have been shown to react with fatty acid thioesters of coenzyme A to form the diacyl phosphatidico. Preliminary work from this laboratory (4) demonstrated the presence of lysophosphatidylinositol in pigeon pancreas and the acylation of this lysophosphatide in the presence of oleic acid, coenzyme A, and adenosine triphosphate.

The present paper is a more detailed study of the acylation of "P-labeled lysophosphatidylinositol. The lysophosphatidylinositol substrate was prepared by the enzymatic decylation of radioactive phosphatidylinositol from pigeon pancreas. A very simple and precise assay method was developed which permits the acylation reaction to be followed. With this method, it has been possible to study the conditions under which the acyl group is transferred from acyl-CoA to lysophosphatidylinositol. Comparisons were also made between the rate of transfer of a saturated (palmityl-) and an unsaturated (oleyl-) CoA derivative. The relative rates of acylation of lysophosphatidylcholine and lysophosphatidylinositol were also compared, and preliminary kinetic studies were undertaken in an attempt to elucidate the mechanism of this reaction.

EXPERIMENTAL PROCEDURE

Analytical Methods—Protein was determined by the method of Lowry et al. (5). Phosphorus was measured by the method of Bartlett (6). Ester content was determined as described by Rapport and Alonzo (7) with methyl oleate as a standard. Inositol was assayed by a modification of the method of Atkin et al. (8), with Saccharomyces carlsbergensis.

To determine the radioactivity in phosphatidylinositol and lysophosphatidylinositol, the lipid extracts were separated by thin layer chromatography on silica gel G with phenol-concentrated ammonia (99:1) as the developing solvent. After chromatography, the plates were dried in an oven at about 50°, sprayed with "Neatan,' and then returned to the oven for about 10 to 20 minutes. Autoradiograms were then prepared, and the spots were carefully cut out and counted. This method is described in more detail elsewhere (9).

Acyl-CoA Derivatives—Oleyl- and palmityl-CoA derivatives were synthesized by the method of Seubert (10) with only minor modifications. The products were purified by chromatography on silicic acid columns as described by Cleland.2 The ratio of thioester absorption at 232 nm to adenine absorption at 260 nm was used as the criterion of purity. Absorption ratios (A232:A260) of 0.53 and 0.56 were obtained for oleyl- and palmityl-CoA, respectively. In some experiments, preparations of these two derivatives which were not purified by chromatography were used. These derivatives had slightly higher A232:A260 ratios. Samples of highly purified oleyl-CoA and palmityl-CoA, kindly provided by Dr. W. W. Cleland and Dr. Hussein Abou-Issa, were also used. All of the preparations of a given fatty acyl-CoA gave reaction velocities which were essentially the same.

Enzyme Preparations—Microsomes were isolated from pigeon pancreas tissue as described by Redman and Hokin (11), except that 0.25 M sucrose was used instead of 0.5 M sucrose. The isolated microsomal pellet was suspended in 4 volumes (based on fresh tissue weight) of 0.25 M sucrose. Aliquots (0.1 ml), corresponding to 25 mg of wet tissue, were employed unless otherwise specified. Freshly isolated microsomes were used within several hours after preparation.

Bee venom was obtained from Sigma Chemical Company and Crotalus adamanteus venom from the California Corporation for Biological Research. Both of these dried venoms were used without further purification as a 0.1% solution in either distilled water or CaCl2 solution.

Preparation of "P-lyosphosphatidylinositol—Pigeon pancreas was chosen as a source of lysophosphatidylinositol, since the phosphtididylinositol from which it is derived attains a very high specific activity in slices incubated with "P in the presence of cholinergic agents (12). The procedure which was followed for the isolation of lysophosphatidylinositol, and typical results which were obtained are given below.

Pigeon pancreas slices (0.91 g) were incubated in 6 ml of the medium of Krebs and Henseleit (13) containing 1 mc of Na2H3PO4 and 1 mM carbamylcholine. After 75 minutes, the slices were removed, combined with 3.1 g of unincubated pigeon pancreas tissue, and homogenized with 25 ml of ice-cold 5% trichloroacetic acid. The resulting precipitate was washed once by suspension in 25 ml of cold 5% trichloroacetic acid. The lipids were extracted by homogenizing the precipitate in 7.5 ml of ethanol, followed by 7.5 ml of chloroform. After standing at room temperature for 10 to 15 minutes, the lipid extract was stirred with 20 ml of ice-cold 0.1 N HCl. The resulting emulsion was centrifuged, and 6.0 ml of the lower chloroform layer were removed; the protein-rich interface and the ethanol-chloroform layer were again extracted with 6.0 ml of chloroform. The con-

* This work was supported by grants from the United Cerebral Palsy Research and Educational Foundation, The Wisconsin Alumni Research Foundation, and the National Institutes of Health.
† Postdoctoral Fellow, National Institute of Neurological Diseases and Blindness. Present address, Department of Biochemistry, Tufts University School of Medicine, Boston 11, Massachusetts.
‡ Research Career Awardee of the National Institutes of Health (Grant 5-K6-GM-1347).
1 Trade name of E. Merck AG, Darmstadt, Germany.
2 W. W. Cleland, personal communication.
This fraction (213 pg of phosphorus) was found to be mainly reducible, the entire operation usually requiring less than 5 hours. An elution diagram was obtained as shown in Fig. 1. The radioactive lysophosphatidylcholine which was isolated and added remained in the upper phase. This procedure partitions the lysophosphatidylinositol into the upper phase (methanol-water) while lysophosphatidylethanolamine and any lysophosphatidylserine distribute into the lower phase (chloroform). Any phosphatidylinositol which was not deacylated would also go into the lower phase. This method will be discussed in greater detail in connection with the assay of phosphatidylinositol and lysophosphatidylinositol (see “Results”).

The upper phase was dried in a vacuum, dissolved in chloroform-methanol (1:1, v/v), and applied to an alumina column, 1 x 5 cm. Chromatography was carried out with the same procedure as shown in Fig. 1. Over 98% of the added activity was recovered in the ethanol-chloroform-water (5:2:2, v/v) eluate. This eluate was concentrated to dryness in a vacuum; the residue was dissolved in chloroform-methanol (1:1, v/v), and the specific activity was determined. The total yield of the lysophosphatidylinositol (based on phosphorus) was 90% of that in the original phosphoinositide fraction. Analyses for ester, phosphorus, and inositol gave ratios which agreed closely with the theoretical ratios of 1:1:1 for lysophosphatidylinositol (1.03:1.00:0.99 and 1.00:1.00:0.99 for two separate preparations). Preparations were chromatographically homogeneous, and essentially 100% of the phosphate and radioactivity were found in one spot in three chromatographic systems, (9, 17, 18).

Preparation of $^{32}$P-Labeled Lysophosphatidylcholine—Guinea pig liver slices (3.0 g) were incubated for 5 hours with 4 mc of $^{32}$P in 10 ml of the medium of Krebs and Henseleit (13). The slices were removed from the medium and combined with 7.5 g of unincubated guinea pig liver. The total lipids were extracted as described above and chromatographed on an alumina column, 3 x 10 cm. The neutral lipids were removed with chloroform, and the phosphatidylcholine fraction was eluted with chloroform-methanol (1:1, v/v). The isolated phosphatidylcholine fraction was dried in a vacuum, dissolved in 100 ml of diethyl ether, and incubated overnight with 1.0 ml of 0.1% Crotaulus adamanteus venom in 0.005 M CaCl$_2$. The cloudy, white precipitate which formed was isolated, and a sample was chromatographed on a silicic acid column as described by Warner and Lands (19). The radioactive lysophosphatidylcholine which was isolated was found to be chromatographically homogeneous in three different systems (9, 17, 18).

Incubation of Pancreas Microssomes—The reaction mixtures (0.50 ml) were incubated in 12-ml centrifuge tubes for 5 minutes at 38°. Unless otherwise specified, each tube contained 0.2 m phosphate buffer, pH 7.4, and 0.05 μmoles of acyl-CoA. “Solutions” of $^{32}$P-labeled lysophosphatidylinositol or $^{32}$P-labeled lysophosphatidylcholine were added. To prepare these solutions, aliquots of the labeled lysophosphatides (dissolved in chloroform-methanol) were taken to dryness in a centrifuge tube immediately before use. A volume of double distilled water sufficient to give a solution of the desired strength was added, and the contents of the tube were mixed vigorously with a Vortex.
mixer. This procedure brought all of the lysophosphatidic into the aqueous phase as a stable, almost water-clear, preparation. The enzyme solution (0.10 ml of pigeon pancreas microsomes in 0.25 M sucrose) was generally added as a last step before incubation.

**Determination of Phosphatidylinositol Synthesis**—The enzymatic reaction was stopped by adding 1.88 ml of chloroform-methanol (1:2, v/v), followed by 0.93 ml of CHCl₃, and finally 0.63 ml of double distilled water. The contents of the tubes were mixed with a Vortex mixer after each addition. The resulting emulsion separated into two phases after centrifugation, and aliquots of the upper and lower phases were plated on planchets and counted. This solvent combination was previously developed for the extraction of fish lipids (10).

**RESULTS**

**Assay of Radioactivity in Phosphatidylinositol and Lysophosphatidylinositol**—Table I shows the distribution of purified radioactive phosphatidylinositol and lysophosphatidylinositol between the upper (methanol-water) and the lower (chloroform) phases at zero time under the reaction conditions employed. It can be seen from the data presented in this table that this is a very precise and simple means of separating these two compounds. Blanks with no acyl-CoA and boiled controls were routinely run to correct for the fraction of the lysophosphatidylinositol radioactivity which is distributed into the chloroform phase (2 to 3% of the total radioactivity added). Blanks were always quite constant within a given experiment. The validity of the partition method as a measure of the lysophosphatidylinositol remaining and phosphatidylinositol formed was checked by chromatographing aliquots of the upper and lower phases by thin layer chromatography on silica gel G with phenol-concentrated ammonia (99:1) according to the method of Redman and Keenan (9). Duplicate analyses agreed closely.

**Table I**

<table>
<thead>
<tr>
<th>Compound added</th>
<th>Per cent of added radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chloroform phase</td>
</tr>
<tr>
<td>Phosphatidylinositol</td>
<td>0.0275 μmole</td>
</tr>
<tr>
<td></td>
<td>0.0550 μmole</td>
</tr>
<tr>
<td>Lysophosphatidylinositol</td>
<td>0.026 μmole</td>
</tr>
<tr>
<td></td>
<td>0.0522 μmole</td>
</tr>
</tbody>
</table>

**Table II**

**Distribution of lysophosphatidylinositol-acylating activity in subcellular fractions of pigeon pancreas**

Each tube contained 0.013 μmole of 32P-labeled lysophosphatidylinositol, 0.2 M phosphate buffer, 0.025 μmole each of palmityl- and oleyl-CoA, and 0.10 ml of the appropriate subcellular fraction equivalent to 7.0 mg of wet tissue in 8.5% sucrose. Final volumes were 0.50 ml. Values given are averages of duplicate determinations.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein (mg/tube)</th>
<th>Phosphatidylinositol (μmole)</th>
<th>Phosphatidylinositol (μmole/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole homogenate</td>
<td>1.090</td>
<td>7.07</td>
<td>7.03</td>
</tr>
<tr>
<td>Nuclear and cell debris</td>
<td>0.292</td>
<td>0.251</td>
<td>1.25</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>0.226</td>
<td>4.40</td>
<td>19.4</td>
</tr>
<tr>
<td>Microsomal</td>
<td>0.180</td>
<td>5.48</td>
<td>29.0</td>
</tr>
<tr>
<td>Supernatant</td>
<td>0.442</td>
<td>0.852</td>
<td>1.83</td>
</tr>
</tbody>
</table>

with microsomal elements (20). However, it is possible that there is inherent mitochondrial activity also.

**Effect of pH**—Fig. 2 shows that the reaction velocity obtained in phosphate buffer increased more or less linearly from pH 5.8 to pH 7.8. The effect of a more alkaline environment was not studied, because of the change in the distribution of phosphatidylinositol between the chloroform and the methanol-water phases of the assay solvent at a pH greater than 8.0.

**Effect of Protein Concentration**—Fig. 3 shows that with the microsomal preparation tested, the rate of lysophosphatidylinositol acylation in the presence of oleyl-CoA was directly proportional to the protein concentration up to about 0.8 mg of protein per ml. A linear relationship between enzyme activity and protein concentration was obtained over this range of protein concentration when lysophosphatidylinositol was acylated in the presence of oleic acid, CoA, and ATP. In most of the experiments reported here the protein concentration was in this range. The specific activities of the various enzyme preparations varied,
FIG. 2 (left). The effect of pH. The pH of the phosphate buffer (0.2 M) added was varied as shown. Each tube contained 0.1 ml of microsomal preparation (0.20 mg of protein), 0.1 μmole of oleyl-CoA per ml, and 0.191 μmole of 32P-labeled lysophosphatidylinositol per ml. Other conditions were as described under “Experimental Procedure.”

FIG. 3 (right). Dependence of reaction on protein concentration. The quantity of microsomal protein per ml of incubation medium was varied as shown. Incubations were carried out in the presence of oleyl-CoA (0.1 μmole per ml) and 32P-labeled lysophosphatidylinositol (0.230 μmole per ml). Other conditions were as described under “Experimental Procedure.”

![Graph showing the effect of pH on reaction velocity](image)

![Graph showing the effect of protein concentration on reaction velocity](image)

TABLE III

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Lyso- phosphatidylinositol added</th>
<th>Lyso-phosphatidylinositol remaining</th>
<th>Change in lyso-phosphatidylinositol</th>
<th>Phosphatidylinositol formed</th>
<th>Change in phosphatidylinositol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>113</td>
<td>02</td>
<td>-21</td>
<td>21</td>
<td>+21</td>
</tr>
<tr>
<td>Minus oleyl-CoA</td>
<td>113</td>
<td>113</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

but most of them gave similar activities on the basis of fresh tissue weight.

**Time Course of Aclylation Reaction**—The formation of phosphatidylinositol from 32P-labeled lysophosphatidylinositol was followed with time, both in the presence of oleic acid, CoA, Mg++, and ATP and in the presence of oleyl-CoA. The results in Fig. 4 indicate that the formation of the CoA thioester must have been a rate-limiting step, since much more phosphatidylinositol was synthesized per unit of time in the presence of oleyl-CoA. The reaction rate, over approximately the first 10 minutes, was linear with relation to oleyl-CoA concentration. In this particular experiment the reaction was considerably slower than in most other experiments, probably because 2.5 times more substrate was used than usual; in a separate experiment this concentration of substrate inhibited the reaction rate. The higher concentration of substrate used here was necessitated by the fact that the reaction was carried on for a much longer time. In another experiment, not shown here, linear reaction rates were obtained over the first 10 minutes with the usual acyl-CoA concentration. It will be noted in Fig. 4 that the reaction did not go to completion. Possible explanations for this are inactivation of the enzyme or side reactions which would make the acyl-CoA limiting at later time intervals. It is unlikely that the leveling off of the curve in Fig. 4 was due to degradation of phosphatidylinositol, since the amount of this product remained constant from 30 to 120 minutes.

**Stoichiometry and Requirement for Fatty Acyl-CoA**—Table III
shows that for each molecule of phosphatidylinositol formed, 1 molecule of lysophosphatidylinositol disappeared. When oleyl-CoA was omitted, no phosphatidylinositol formation could be detected. These data indicate that the following reaction took place.

\[ \text{Lysophosphatidylinositol} + \text{oleyl-CoA} \rightarrow \text{phosphatidylinositol} + \text{CoA} \quad (1) \]

It has been suggested by Erbland and Marinetti (21) and Kokke et al. (22) that the following reaction is catalyzed by enzymes present in liver and yeast.

\[ 2 \text{Lysophosphatidylcholine} \rightarrow \text{phosphatidylcholine} + \text{glycerylphosphorylcholine} \quad (2) \]

The fact that phosphatidylinositol was not formed in the absence of acyl-CoA indicates that this type of reaction did not take place under our conditions. There was also no formation of phosphatidylcholine from lysophosphatidylcholine under our conditions when fatty acyl-CoA was omitted (see Figs. 6 and 7).

Attempts to Demonstrate Glycerylphosphorylinositol Acylation—Experiments were carried out to see if glycerylphosphorylinositol could be acylated in the same manner as α-glycerophosphate (23). Glycerylphosphorylinositol, 32P-labeled, was prepared by the mild alkaline hydrolysis (24) of 32P-labeled phosphatidylinositol. The product was purified by ion exchange chromatography (25). Samples of 32P-labeled glycerylphosphorylinositol of high specific activity were incubated with pancreas microsomes for periods up to 2 hours. No radioactive phosphatidylinositol or lysophosphatidylinositol could be detected in these experiments.

Effect of Varying Lysophosphatidylinositol Concentration in Presence of Oleyl-CoA or Palmityl-CoA—The influence of increasing concentrations of lysophosphatidylinositol on the rates of the acylation reaction were studied in the presence of fixed quantities of either oleyl- or palmityl-CoA (Fig. 5). The initial velocities obtained in the presence of oleyl-CoA were more than twice those obtained with palmityl-CoA at all concentrations tested, indicating that the unsaturated fatty acid was the preferred substrate.

Double reciprocal plots of the data in Fig. 5 gave values of $1.5 \times 10^{-4}$ M (1.1 and $1.8 \times 10^{-4}$ M were obtained in two other experiments) and $0.7 \times 10^{-4}$ M as the concentrations of lysophosphatidylinositol.
Effects of increasing the oleyl-CoA and palmitoyl-CoA concentrations on the rate of acylation of either lysophosphatidylinositol or lysophosphatidylcholine are shown in Fig. 6. The most highly purified acyl-CoA derivatives were employed for this experiment, and judged by their 

\[
K_m = \frac{V_{max}}{K_{cat}}
\]

values for the acyl-CoA derivatives were determined. In the presence of lysophosphatidylcholine the values were $5.6 \times 10^{-4} M$ for oleyl-CoA and $3.1 \times 10^{-4} M$ for palmitoyl-CoA. In the presence of lysophosphatidylcholine the apparent

\[
K_m = \frac{V_{max}}{K_{cat}}
\]

values were $2.7 \times 10^{-4}$ and $1.9 \times 10^{-4} M$ for oleyl- and palmitoyl-CoA, respectively. These values were determined in the presence of concentrations of the lysophosphatides which were optimum but not saturating (higher concentrations produced inhibitions). The acylation of lysophosphatidylcholine was inhibited at lower concentrations. These studies were also carried out with higher concentrations of oleyl-CoA and palmitoyl-CoA in a separate experiment. Concentrations of over $1 \times 10^{-4}$ M of either oleyl or palmitoyl CoA did not give linear double reciprocal plots. The inhibitions observed, however, were not as marked as those observed in the presence of excess lysophosphatidylcholine.

Effects of Varying Concentration of Lysophosphatidylinositol or Lysophosphatidylcholine—Fig. 7 shows the effect of increasing concentrations of lysophosphatidylinositol and lysophosphatidylcholine on the synthesis of phosphatidylinositol and phosphatidylcholine in the same experiment. The results show that over a wide range of concentrations, the acylation of lysophosphatidylcholine proceeded at more than twice the rate of that of lysophosphatidylcholine.

Another conclusion which can be drawn from these data is that the rapid hydrolytic breakdown of oleyl-CoA described by Lands and Merkl (27) could not be a significant factor in measuring initial velocities under the conditions of these experiments. It will be noted in Fig. 7 that as much as 70% of the acyl-CoA had reestered with lysophosphatidylcholine at 5 minutes. If the reaction had been carried out for a longer time, more acyl-CoA (perhaps close to 100%) would have been incorporated into lecithin. The acyl-CoA must have been saturating during the first 5 minutes of acylation of lysophosphatidylinositol, however, since excellent Lineweaver-Burk (26) plots were obtained from the data in Fig. 7.

DISCUSSION

In the present paper we have provided evidence for an enzyme in pigeon pancreas tissue which catalyzes the acylation of lysophosphatidylinositol in the presence of coenzyme A thioesters of the fatty acids to form phosphatidylinositol. This system was shown to be most highly concentrated in the microsomal fraction. The development of a simple and precise assay procedure based on differences in the solubilities of lysophosphatidylinositol and phosphatidylinositol in a biphasic system of chloroform-methanol-water (1:1:0.9, v/v) aided greatly in these studies. It was possible to show that all of the detectable phosphatidylinositol synthesized was obtained as a result of the transfer of the acyl group from acyl-CoA to lysophosphatidylinositol. This reaction is shown in Equation 1 (see “Results”). No evidence for a trans-esterification reaction between the phosphatides or lysophosphatides was obtained.

Lands and Merkl (27) showed that the rate at which the acylation of lysophosphatidylcholine takes place is dependent on the nature of the fatty acid transferred as well as on the position of the acceptor group of the lysophosphatide molecule. In the present experiments the lysophosphatidylinositol which was employed as a substrate was presumably α'-acyllysophosphatidyl-}

\[
10^{-4}
\]

inositol. This assumption is based on the method of preparation, which involved the deacylation of phosphatidylinositol by bee venom phospholipase A. This enzyme has been demonstrated to hydrolyze specifically the β-esterified fatty acids from those phospholipids which have been studied (27–31). In experiments not shown here linoleoyl-CoA was found to acylate lysophosphatidylcholine as rapidly as or more rapidly than oleyl-CoA over a range of concentrations. Stearyl-CoA was compared with oleyl-CoA and palmitoyl-CoA at a single concentration and was found to be 75% as effective as palmitoyl-CoA. The activities of these various acyl-CoA derivatives in acylating lysophosphatidylinositol parallel in a general way those reported by Lands and Merkl (20) for the acylation of lysophosphatidylcholine.

Some kinetic studies were carried out in order to determine the concentrations of substrates at which the maximal velocities are attained. Since two substrates are involved in the acylation reaction, it was necessary to hold the concentration of one substrate constant and to vary the other. These studies were complicated by the fact that both substrates were inhibitory near their estimated $K_m$ values, especially the lysophosphatidylcholine. This was due to their $K_m$ values being higher than the concentration of the nonvaried substrate was fixed at an optimum level (higher concentrations resulted in inhibition). The apparent

\[
K_m = \frac{V_{max}}{K_{cat}}
\]

values obtained under these conditions for oleyl- and palmitoyl-CoA were about 10 times higher with lysophosphatidylcholine as acyl acceptor than with phosphatidylinositol as the acceptor.

A comparison of the relative rates of synthesis of the two phosphatides (as shown in Fig. 7) was also made. The highest velocities which were attained were 14.8 mmol per minute per mg of protein for phosphatidylcholine formation and 6.8 mmol per minute per mg of protein for phosphatidylinositol formation.

The fact that the nature of the acceptor apparently influences the $K_m$ values of the fatty acyl-CoA derivatives and that lysophosphatidylcholine is acylated twice as fast as lysophosphatidylinositol does not necessarily mean that different enzymes are required for these reactions. The obvious way to determine
whether the same enzyme is responsible for both reactions is to see if the acylation of one substrate is inhibited by the presence of the other. The acylation of \(^{32}P\)-labeled lysophosphatidyl-
inositol was studied in the presence and absence of unlabeled lysophosphatidylcholine, but because of the complexity of this system, the results were inconclusive. It was clear, however, that the presence of lysophosphatidylcholine did not produce a simple competitive inhibition of lysophosphatidylinositol acylation. More intricate kinetic experiments will have to be conducted to determine whether the same enzyme is responsible for the acylation of both lysophosphatides. A simpler approach might be to attempt to separate the two activities by enzyme purification.

In contrast to the work of Brockerhoff (32), it has been found in this laboratory that the polyunsaturated fatty acids of phosphatidylinositol from pigeon pancreas are specifically localized in one position on the glycerol moiety, presumably the \(\beta\) position.\(^3\) The results of Noda and Song (33) for soybean phosphatidyl-
inositol point in a similar direction. On the basis of the data presented here it appears likely that independent turnover of the \(3a\)P-labeled lysophosphatidyl-
inositol was studied in the presence and absence of unlabeled lysophosphatidylcholine, but because of the complexity of this system, the results were inconclusive. It was clear, however, that the presence of lysophosphatidylcholine did not produce a simple competitive inhibition of lysophosphatidylinositol acylation. More intricate kinetic experiments will have to be conducted to determine whether the same enzyme is responsible for both reactions is to attempt to separate the two activities by enzyme purification.

SUMMARY

Pigeon pancreas microsomal and mitochondrial fractions were found to catalyze the formation of phosphatidylinositol from fatty acid thiester of coenzyme A and lysophosphatidylcholine. A simple assay method was developed for following this reaction. Kinetic parameters of the acylation reaction were studied. It was found that oleyl-coenzyme A was a better substrate than palmitoyl coenzyme A. The rates of acylation of lysophosphatidylcholine and lysophosphatidylcholine were compared, and lysophosphatidylcholine was found to be acylated more rapidly than lysophosphatidylcholine irrespective of the acyl coenzyme A derivatives tested.

Acknowledgments—The authors wish to thank Dr. Leonard Fahien and Dr. M. R. Hokin for their valuable suggestions, and Mr. Alphonse Ingenito for technical assistance.

REFERENCES


29. TATTHE, N. H., J. Lipid Research, 1, 60 (1959).
The Enzymatic Acylation of Lysophosphatidylinositol
R. W. Keenan and Lowell E. Hokin


Access the most updated version of this article at
http://www.jbc.org/content/239/7/2123.citation

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/239/7/2123.citation.full.html#ref-list-1