The Enzymatic Acylation of Lysophosphatidylinositol*

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Lysophosphatidylcholine (1), lysophosphatidylethanolamine (2), and lysophosphaticid acid (3) have been shown to react with fatty acid thioesters of coenzyme A to form the diacyl phosphatidylethanolamine. 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 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 (palmitoyl-) and an unsaturated (oleyl-) CoA derivative.

The present 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 80°C, sprayed with Ncatal, 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—Oleoyl- and palmitoyl-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. The ratio of thioester absorption at 232 mμ to adenine absorption at 260 mμ was used as the criterion of purity. Absorption ratios (A232:260) of 0.51 and 0.56 were obtained for oleoyl- and palmitoyl-CoA, respectively. In some experiments, preparations of these two derivatives which were not purified by chromatography were used. These derivatives had slightly higher A232:260 ratios. Samples of highly purified oleoyl-CoA and palmitoyl-CoA, kindly provided by Dr. R. 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.

Preparation of [3P]-Lysophosphatidylinositol—Pigeon pancreas was chosen as a source of lysophosphatidylinositol, since the phosphatidylinositol from which it is derived attains a very high specific activity in slices incubated with 32P in the presence of cholinerogic agents (1). 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 me of Na2HPO4 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 with 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 com-
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 radioactivity (9) in the acetone precipitate was enriched 2- to 3-fold over 90%. The dark brown material present in the lipid extract with respect to total phospholipid phosphorus, with a yield of 80% of the phosphatidylinositol (determined by thin layer chromatography) was collected by centrifugation. The radioactivity which formed was isolated, and a sample was chromatographed on an alumina column with the solvents described by Hanahan (14). Phosphate, O—O; radioactivity, O—O.

**Peak I.** Phosphatidylcholine; **II.** Phosphatidylethanolamine and phosphatidylserine; **III.** Phosphatidylinositol and lysophosphatidylethanolamine. Phosphatidic acid was tightly bound to the alumina chromatography was quite rapid and extremely reproducible. The phospholipids were then eluted from the alumina column with the solvents described by Hanahan (14). Phosphate, O—O; radioactivity, O—O.

Fig. 1. The chromatography of pigeon pancreas phospholipid on alumina. Phosphate, O—O; radioactivity, O—O. **Peak I.** Phosphatidylcholine; **II.** Phosphatidylethanolamine and phosphatidylserine; **III.** Phosphatidylinositol and lysophosphatidylethanolamine. Phosphatidic acid was tightly bound to the column. The solvents used were chloroform-methanol (1:1, v/v) and ethanol-chloroform-water (5:2:2, v/v). Arrows indicate the points where solvents were changed. The volume of each fraction was 5.0 ml.

The dried phosphoinositide fraction was dissolved in 50 ml of diethyl ether, and 0.5 ml of 0.1% bee venom in double distilled water was added (15). The flask containing this sample was shaken overnight at room temperature. At the end of this time, absolute ethanol was added and the contents of the flask were concentrated to dryness. The dried residue was dissolved in chloroform-methanol (1:1, v/v), transferred to a 40-ml centrifuge tube, and made up to 20 ml with this solvent. Double distilled water (9.0 ml) was added, and the contents of the tube were thoroughly mixed. After centrifugation the lower phase, which is essentially pure chloroform (16), was removed and replaced with the same volume of chloroform. The washing operation was repeated twice. Over 96% of the radioactivity initially 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. Fractions for ester, phosphorus, and inositol gave ratios which agreed closely with the theoretical ratios of 1:1:1 for lysophosphatidylcholine and lysophosphatidylcholine.

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 32P-Labeled Lysophosphatidylcholine—Guinea pig liver slices (3.0 g) were incubated for 5 hours with 4 mc of 32P 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% Crotalus adamanteus venom in 0.005 M CaCl2. 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 Microsomes—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 32P-labeled lysophosphatidylcholine or 32P-labeled lysophosphatidylcholine were added. To prepare these solutions, aliquots of the labeled lysophosphatidylcholines (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 lysophosphatidylcholines 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 Phosphatidylcholine Synthesis**—The enzymatic reaction was stopped by adding 1.88 ml of chloroform-methanol (1:2, v/v), followed by 0.63 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 Phosphatidylcholine and Lysophosphatidylcholine** Table I shows the distribution of purified radioactive phosphatidylcholine and lysophosphatidylcholine 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 run with no acyl-CoA and boiled controls were routinely run to correct for the fraction of the lysophosphatidylcholine 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 lysophosphatidylcholine remaining and phosphatidylcholine 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). The reaction velocities which were determined are given in Table II as mmol/mg protein.

**Subcellular Localization of Lysophosphatidylcholine Acylase Activity**—Samples of the various subcellular fractions from equivalent amounts of fresh tissue were compared for their ability to acylate phosphatidylcholine. The data given in Table II show that the greatest total activity and the highest specific activity were present in the mitochondrial fractions, although the microsomal fraction was also very active. The microsomal fraction isolated by this procedure is known to be relatively pure, while the mitochondrial fraction is contaminated 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 phosphatidylcholine 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 lysophosphatidylcholine 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 lysophosphatidylcholine 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 (620 µg of protein), 0.1 µmole of oleyl-CoA per ml, and 0.191 pmole 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.”

**Stoichiometry and Requirement for Fatty Acyl-CoA—Table III**

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Lyso-</th>
<th>Phos-</th>
<th>Change in</th>
<th>change in</th>
<th>change in</th>
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<tr>
<td></td>
<td>phatidylino-</td>
<td>phatidylinositol</td>
<td>lysophatidylinositol</td>
<td>phosphatidylinositol</td>
<td>phosphatidylinositol</td>
</tr>
<tr>
<td>Plus oleyl-CoA</td>
<td>113</td>
<td>0</td>
<td>21</td>
<td>0</td>
<td>+21</td>
</tr>
<tr>
<td>Minus oleyl-CoA</td>
<td>113</td>
<td>113</td>
<td>0</td>
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but most of them gave similar activities on the basis of fresh tissue weight.

**Time Course of Acylation 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.

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

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, \(^{32}\)P-labeled, was prepared by the mild alkaline hydrolysis (24) of \(^{32}\)P-labeled phosphatidylinositol. The product was purified by ion exchange chromatography (25). Samples of \(^{32}\)P-labeled glycerylphosphorylinositol of high specific activity were incubated with pancreas microsomes in the presence of oleyl-CoA 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 x 10\(^{-4}\) M (1.1 and 1.8 x 10\(^{-4}\) M were obtained in two other experiments) and 0.7 x 10\(^{-4}\) M as the 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.
phosphatidylinositol at which half-maximal velocities were obtained in the presence of oleyl- and palmitoyl-CoA, respectively. The concentrations of the acyl-CoA derivatives which were employed for these experiments were approximately twice their $K_m$ values; higher concentrations did not lead to increased reaction velocities. The effects of lysophosphatidylinositol concentrations higher than those shown in Fig. 4 were also studied. When the concentration of lysophosphatidylinositol reached $3 \times 10^{-4}$ M or higher, the Lineweaver-Burk (26) plots departed from linearity, indicating inhibition by substrate. At concentrations of lysophosphatidylinositol above $8 \times 10^{-4}$ M the acylation reaction was completely inhibited. This inhibition may have been produced by a detergent action of lysophosphatidylinositol.

Effects of Varying Acyl-CoA Concentrations on Acylation of Lysophosphatidylinositol and Lysophosphatidylcholine—The 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 $A_{235}:A_{260}$ ratios, they were essentially 100% pure. The greater effectiveness of oleyl-CoA for acylating both lyso derivatives is in accord with the data in Fig. 5. It is also apparent that lysophosphatidylcholine was acylated about twice as fast as lysophosphatidylinositol at all concentrations of acyl-CoA tested. These data (Fig. 6) were also plotted by the method of Lineweaver and Burk (26). The apparent $K_m$ values for the acyl-CoA derivatives were determined. In the presence of lysophosphatidylcholine the values were $5.3 \times 10^{-6}$ M for oleyl-CoA and $3.1 \times 10^{-6}$ M for palmitoyl-CoA. In the presence of lysophosphatidylinositol the apparent $K_m$ values were $2.7 \times 10^{-4}$ and $1.0 \times 10^{-4}$ 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 lysophosphatidylinositol was investigated with higher concentrations of oleyl- 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 lysophosphatidylinositol.

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 lysophosphatidylinositol.

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 reacetylated 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 phosphatidylglycerol. 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 solubilibilities of lysophosphatidylglycerol and phosphatidylglycerol 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 phosphatidylglycerol 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 transesterification 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 lysophosphatidylglycerol which was employed as a substrate was presumably α',ω'-lysophosphatidylglycerol. 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 linoleyl-CoA was found to acylate lysophosphatidylglycerol 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 lysophosphatidylglycerol 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 half-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 lysophosphatidylglycerol. For this reason, apparent $K_m$ values were determined from the linear range of the double reciprocal plots. The concentration of the nonvaried substrate was fixed at an optimum level (higher concentrations resulted in inhibition). The apparent $K_m$ values obtained under these conditions for oleyl- and palmitoyl-CoA were about 10 times higher with lysophosphatidylcholine as acyl acceptor than with lysophosphatidylglycerol 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 μmoles per minute per mg of protein for phosphatidylcholine formation and 6.8 μmoles per minute per mg of protein for phosphatidylglycerol 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 lysophosphatidylglycerol 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 \(^{3}P\)-labeled lysophosphatidylinositol was found to catalyze the formation of phosphatidylinositol from pigeon pancreas are specifically localized in one position on the glycerol moiety, presumably the \(\beta\) position. The results of Noda and Song (33) for soybean phosphatidylcholine and lysophosphatidylinositol were compared, and lysophosphatidylcholine was found to be acylated more rapidly than lysophosphatidylinositol irrespective of the acyl position on the glycerol moiety, presumably the \(\beta\) position.3

In contrast to the work of Brockerhoff (32), it has been found in pigeon pancreas microsomal and mitochondrial fractions 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

**SUMMARY**

Pigeon pancreas microsomal and mitochondrial fractions were found to catalyze the formation of phosphatidylinositol from fatty acid thioesters 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 phosphatidylinositol were compared, and lysophosphatidylcholine was found to be acylated more rapidly than lysophosphatidylcholine irrespective of the acyl coenzyme A derivatives tested.

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