The Synthesis of Phosphatidic Acid from Diglyceride and Adenosine Triphosphate in Extracts of Brain Microsomes*

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Previous studies in several laboratories have shown that phosphatidic acid becomes labeled with P$^{32}$ in cell-free preparations of brain during oxidative phosphorylation (1-3). Kornberg and Price (4) found an enzyme in liver which catalyzes the synthesis of phosphatidic acid from $\alpha$-glycerophosphate and coenzyme A in the supernatant fluid was carefully collected. Where indicated, these preparations were dialyzed against a mixture of equal parts of isotonic sucrose and 0.012 M sodium deoxycholate in 0.04 M potassium glycylglycine, pH 7.0, for 16 hours in the cold room. The soluble extracts were stored at $-20^\circ$.

The preparations were incubated in 12-ml Servall centrifuge tubes for 1 hour at 38$^\circ$ with shaking in a Dubnoff metabolic incubator. At the end of the incubation period, 5 ml of 5 per cent trichloroacetic acid were added to each tube and the material was centrifuged. In the phosphatase experiments, orthophosphate in the supernatant fluid was determined by the method of Berenblum and Chain (5). In the radioautographic experiments, the precipitate was treated to remove contaminating P$^{32}$, and the lipides were extracted and purified as described previously (6). Phosphatidic acid was isolated by chromatography either on silicic acid-impregnated paper (prepared with reagent grade silicic acid) with diisobutyl ketone-acetic acid-water (40:20:5) as the solvent (7, 8) or on Schleicher and Schuell chromatography paper No. 2045B with diisobutyl ketone-methyl isobutyl ketone-methyl ethyl ketone-chloroform-formic acid (98 per cent)-water (30:26:10:110:30:3) as the solvent (9). The amount of phosphatidic acid present in the extracts was insufficient to be detected on the chromatograms by staining. In order to avoid the delay of autoradiography, carrier cabbage phosphatidic acid (50 to 100 $\mu$g.) was added to each lipide sample before chromatography, and the spots were then located by staining with Rhodamine G (10). The radioactivities of the total lipide extracts and the phospholipide spots on the chromatograms were determined as described previously (6).

Lipide extracts were digested with $\text{H}_2\text{SO}_4$ and $\text{H}_2\text{O}_2$ for determination of total phospholipide phosphorus. Orthophosphate was then estimated either by the method of Fiske and SubbaRow (11) or by the method of Berenblum and Chain (5).

The quantity of esterified phosphate which remained after incubation was determined by shaking a sample of the trichloroacetic acid supernatant fluid with ammonium molybdate, dilute $\text{H}_2\text{SO}_4$, and isobutanol by the method of Berenblum and Chain (5) and counting aliquots of the aqueous phase. This esterified phosphate represented the maximal amount of ATP which could still be present.

Radioactive Materials—$\alpha$-Glycero-$P^{32}$ was synthesized as described by McMurray et al. (2) but on a scale one hundredth as great as that used by these authors; proportionately 10 times more ortho-$P^{32}$ was used to give material of higher specific activity. To remove ortho-$P^{32}$, carrier orthophosphate was added after the reaction was complete and the orthophosphate was then precipitated by the addition of 0.1 M MgCl$_2$ in 1 M NH$_4$Cl, made alkaline with NH$_2$OH. After standing overnight in the cold, the

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precipitate was removed by centrifugation. This treatment was repeated on the supernatant fluid. The final supernatant fluid was passed over Dowex 50 (H+ form) to remove magnesium and ammonium ions, and the eluate and washings were dried in a vacuum. The maximal specific activity of the glycerol-\(^{32}\)P was 3 × 10^6 c.p.m. per \(\mu\)mole.

Carbamyl-P\(^{32}\) was synthesized either as the lithium salt by the method of Jones et al. (12) or as the ammonium salt by a modification of this method; it had a maximal specific activity of 1.5 × 10^6 c.p.m. per \(\mu\)mole.

ATP\(^{32}\) labeled in the \(\gamma\)-phosphate was synthesized from carbamyl-P\(^{32}\) by a method based on a reversal of the carbamyl kinase reaction described by Jones et al. (12); it had a maximal specific activity of 5 × 10^5 c.p.m. per \(\mu\)mole.

Special Chemicals—Cabbage phosphatidic acid was prepared and purified according to the method of Chibnall and Chanon (13, 14). It was precipitated as the sodium salt from an ethanolic solution of the free acid. Care was taken, in adding ethanolic NaOH, not to allow the pH of the solution to rise higher than about 6.8; in solutions more alkaline than this the material tended to turn brown and become inactive.

To prepare diglyceride, an aqueous solution of sodium phosphatidic acid was incubated at 37°C with prostatic phosphomonoesterase in 0.04 M acetate buffer, pH 5.3, until more than 90 per cent of the phosphate had been liberated as orthophosphate. The diglyceride was then extracted in chloroform-ethanol (1:1), and the solvent was removed in a vacuum. The residue, which was a colorless oil at room temperature, was taken up in 0.012 M sodium deoxycholate. This residue contained less than 5 per cent of the phosphate of the original material.

Brain lecithin was prepared from calf brain by first extracting with acetone and then with ethanol; the ethanol extract was dried in a vacuum to give crude lecithin (15). This was purified on an alumina column by the method of Hanahan et al. (16). α,β-Diglyceride was prepared from the purified brain lecithin by the action of lecithinase D from Clostridium perfringens Type A toxin (kindly supplied by Dr. W. S. Hammond of the Lederle Laboratories) (17).

\(1\)-Palmitoyl, 2-oleyl diglyceride was a gift of Dr. Eugene Kennedy. L-α( Distearoyl)phosphoric acid was a gift of Dr. Eric Baer.

α-Glycerophosphate was obtained from the Eastern Chemical Corporation, Newark, New Jersey. Streptococcus faecalis extracts containing carbamyl klnase were a gift of Dr. Robert Metzgenberg. The units given are those defined by Metzenberg et al. (18). Prostatic acid phosphomonoesterase was a gift of Dr. Charles Heidelberger.

RESULTS

Labeling of Phospholipides on Incubation of Brain Microsomes with Substrate Concentrations of ATP\(^{32}\)—When microsomes were incubated with substrate levels of ATP\(^{32}\), the total lipid extract became weakly labeled. Autoradiograms of chromatograms of the total lipid extract required exposures of 1 month before phosphatidic spots could be seen. Phosphatidic acid was the major radioactive spot. Another faintly radioactive spot with the relative position and staining properties (with Rhodamine G) of phosphoinositide could be faintly seen. The incorporation of P\(^{32}\) from ATP\(^{32}\) into phosphatidic acid was low by comparison with the incorporation of P\(^{32}\) into this phosphatide under conditions of oxidative phosphorylation (3). This was probably due to the very high ATPase activity present in the microsomes; based on measurements of esterified phosphate after 1 hour of incubation, less than 1 per cent of the added ATP was still present in the incubation mixture. Addition of NaN\(_3\) inhibited the breakdown of ATP somewhat and increased the incorporation of P\(^{32}\) into phosphatidic acid about 5-fold.

Incorporation of P\(^{32}\) into Phospholipides in Microsomes in Presence of ATP\(^{32}\)-generating System—An ATP\(^{32}\)-generating system was sought which would provide a steady state level of ATP\(^{32}\). A system found to be particularly useful for this purpose was the reaction catalyzed by carboxykinase from S. faecalis in which ATP\(^{32}\) is formed from carbamyl-P\(^{32}\) and ADP (12). It has recently come to our notice that Weiss et al. (19) have used a similar system for the generation of nonradioactive ATP.

When brain microsomes were incubated with this ATP\(^{32}\)-generating system, under optimal conditions the incorporation of P\(^{32}\) into phosphatidic acid was 10 to 15 times greater than that observed with substrate levels of ATP\(^{32}\). As in the experiments in which substrate concentrations of ATP\(^{32}\) were used, the main radioactive spot on the chromatograms was phosphatidic acid. The faint radioactive spot believed to be phosphoinositide was also seen on the autoradiograms. Under these conditions, an average of 55 per cent (range, 40 to 68 per cent in 16 experiments) of the radioactivity of the total lipides was recovered in the phosphatidic acid spot.

Increasing the concentration of carbamyl-P\(^{32}\) caused an increase in the incorporation of P\(^{32}\) into phosphatidic acid up to a near maximal concentration of 0.005 M; maximal incorporation of P\(^{32}\) into phosphatidic acid was observed with an ADP concentration of 0.0002 M.

Phosphatidic acid was formed in the mitochondrial fraction at a rate which was about one-third to one-sixth as great as that in the microsomal fraction. However, the quantity of total phospholipide phosphorus was less by a similar factor, which suggests that on the basis of their lipid content, the two fractions have approximately equal capacity to synthesize phosphatidic acid from ATP\(^{32}\).

Effect of Adding Deoxycholate to Microsomes—Addition of deoxycholate to the microsome suspensions had marked effects on the synthesis of phosphatidic acid. At concentrations between 5.8 × 10^{-4} M and 3 × 10^{-4} M there was consistently about a 30 per cent inhibition of P\(^{32}\) incorporation into phosphatidic acid. Between 4 × 10^{-4} M and 2.2 × 10^{-3} M the incorporation was greatly stimulated. At 3.6 × 10^{-3} M the activation peak had been passed and at 5.4 × 10^{-2} M the incorporation was 50 per cent less than the control (Fig. 1).

In routine experiments in which deoxycholate-treated microsomes were used, the final concentration of deoxycholate in the incubation mixture was 2.7 × 10^{-3} M, which is near the peak of the activation curve. The activation seems to be somewhat specific for phosphatidic acid synthesis, since under these conditions an average of 75 per cent (range, 64 to 82 per cent in six experiments) of the radioactivity in the total lipides was recovered in the phosphatidic acid spots compared with 55 per cent in the untreated microsomes.

In contrast to the activation of phosphatidic acid synthesis, deoxycholate inhibited the incorporation of P\(^{32}\) into phosphoinositide.

Substrates for Phosphatidic Acid Synthesis—When an aqueous

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solution of cabbage phosphatidic acid was added to intact microsomes, there was no increased incorporation of P32 into phosphatidic acid. However, in the presence of even very low concentrations of deoxycholate (2.9 x 10^{-4} M), addition of cabbage phosphatidic acid increased the incorporation of P32 into the microsomal suspensions (Table I). Kennedy and Weiss (20) found that suspensions of lipides which were suspected of being precursors of a diglyceride moiety for lecithin synthesis were without consistent effect in particulate fractions of liver until surface active agents such as bile salts, digitonin, or Tween 20 were added to the suspensions.

When microsomes treated with 2.7 x 10^{-3} M deoxycholate were centrifuged at 105,000 X g for 30 minutes, the incorporation of P32 into phosphatidic acid in the supernatant fluid (soluble extract) in the absence of added substrate was about 30 per cent of the incorporation in the uncentrifuged deoxycholate-treated suspensions. This was due to a more limiting concentration of substrate in the extract, since in the presence of 0.002 M cabbage phosphatidic acid, the incorporation of P32 into phosphatidic acid in the soluble extracts was greatly increased and was about the same as in the uncentrifuged, treated microsomes (Table I). These results indicate that the enzymes for phosphatidic acid synthesis can be solubilized by deoxycholate treatment under these conditions.

By far the most effective substrate for phosphatidic acid synthesis in the soluble extracts was found to be diglyceride prepared from cabbage phosphatidic acid. The optimal concentration of this diglyceride was about 0.002 M (Fig. 2). At this concentration there was more than a 50-fold increase in the amount of phosphatidic acid synthesized as compared with the control.

The stimulatory effect of diglyceride on phosphatidic acid synthesis was not due to hydrolysis to free fatty acids, since a mixture of free fatty acids in the approximate proportions found in cabbage phosphatidic acid (14), plus glycerol, was less than one-thirtieth as effective as an equivalent amount of diglyceride, although the mixture gave some increase in synthesis. The stimulatory effect of cabbage phosphatidic acid is presumably due to an enzyme which can remove phosphate from cabbage phosphatidic acid (see below) providing diglyceride substrate. Optimal amounts of cabbage phosphatidic acid were not nearly as effective as a substrate as were optimal amounts of diglyceride.

Diglyceride prepared from brain lecithin gave a 3-fold increase in phosphatidic acid synthesis. 1-Palmitoyl,2-oleyl diglyceride

Table I

| Enzyme preparation | Other additions | Phosphatidic acid synthesized
|--------------------|----------------|-----------------------------
| Deoxycholate | Cabbage phosphatidic acid |
| Experiment 1 | | |
| Microsomes | | 0.075 |
| Experiment 2 | | 0.006 |
| Microsomes | | 0.09 |
| Microsomes | 2.9 x 10^{-4} | 0.0009 |
| Microsomes | 2.9 x 10^{-4} | 0.002 |
| Experiment 3 | | 1.53 |
| Microsomes | 2.7 x 10^{-3} | 0.72 |
| Soluble extract | 2.7 x 10^{-3} | 0.27 |
| Soluble extract | 2.7 x 10^{-3} | 4.00 |

Fig. 1. The effect of deoxycholate on phosphatidic acid synthesis from ATP32 in brain microsomal suspensions. Aliquots (0.2 ml.) of the microsomal suspensions were added to 0.2-ml aliquots of an incubation mixture to give the following concentrations in the final medium: 0.1 M sucrose; 0.1 M sodium glycyglycine, pH 6.5; 0.002 M MgSO4; 0.0005 M ADP; 0.004 M lithium carbamyl-P32; carbamyl kinase, 0.3 units per ml.; and sodium deoxycholate as indicated. Incubations were carried out for 1 hour at 37° with shaking. The amount of P32 labeled phosphatidic acid is that obtained from an aliquot which represented one-fifteenth of the total microsomal yield from the cerebral hemispheres of one guinea pig.

Fig. 2. Phosphatidic acid synthesis in soluble extracts of deoxycholate-treated brain microsomes in the presence of increasing concentrations of diglyceride from cabbage phosphatidic acid. Aliquots (0.1 ml.) of a soluble extract were added to 0.1-ml aliquots of an incubation mixture to give the following concentrations in the final medium: 0.05 M sucrose; 0.0027 M sodium deoxycholate; 0.1 M potassium glycyglycine, pH 6.5; 0.002 M MgSO4; 0.0005 M ADP; 0.004 M lithium carbamyl-P32; carbamyl kinase, 0.3 units per ml.; and diglyceride from cabbage phosphatidic acid as indicated. Incubations were carried out for 1 hour at 37° with shaking. The amount of P32 labeled phosphatidic acid is that obtained from an aliquot which represented one-fifteenth of the total yield of soluble extract from the microsomes of the cerebral hemispheres of one guinea pig.
The fatty acids were added as the sodium salts. The final concentration of diglyceride and phosphatidic acid substrates was 0.002 M; the final concentration of fatty acids was 0.004 M except where otherwise indicated. Glycerol (0.002 M) was added with the fatty acids. Other conditions of incubation were as in Fig. 2.

In the absence of added substrate, an average of 52 per cent (range, 30 to 76 per cent in 10 experiments) of the radioactivity of the total lipide fraction was recovered in the phosphatidic acid spot. When allowance is made for loss of phosphatidic acid on chromatography (21), this indicates that the added substrate did not appreciably increase the incorporation of P\(^{32}\) into lipides other than phosphatidic acid under these conditions.

The phosphatidic acid spot increased in size and intensity when increasing amounts of cabbage phosphatidic acid were added to the incubation system. The radioactive spot on the autoradiograms coincided exactly with the stained spot which was seen with larger amounts of cabbage phosphatidic acid, indicating that the substrate was being specifically converted to P\(^{32}\)-labeled phosphatidic acid.

Factors Required for Phosphatidic Acid Synthesis in Soluble Extracts—The factors required for the ATP\(^{32}\)-generating system are carbamyl-\(P^{32}\), ADP, carbamate kinase, and magnesium ions; in addition, NaF is necessary to prevent rapid hydrolysis of the ATP\(^{32}\) formed. The complete system for phosphatidic acid synthesis in the dialyzed soluble fractions of deoxycholate-treated brain microsomes consisted of these factors plus appropriate diglyceride. Omission of any one of these factors reduced to a very low level the incorporation of P\(^{32}\) into phosphatidic acid (Table III).

The maximal concentration of ADP in this system was about 0.0002 M (Table IV). Addition of CDP, UDP, or GDP did not give any increase in the amount of phosphatidic acid synthesized; there was some inhibition of P\(^{32}\) incorporation into phosphatidic acid in the presence of GDP (Table IV).

Incorporation of Glycero-P\(^{32}\) into Phosphatidic Acid in Brain Microsomes and Soluble Extracts. When soluble extracts of deoxycholate-treated brain microsomes were incubated with a non-radioactive ATP-generating system and \(\alpha\)-glycero-P\(^{32}\), the amount of phosphatidic acid synthesized from glycero-P\(^{32}\) was less than one-hundredth of that formed from ATP\(^{32}\) in the absence of added substrate, and less than .0005 of that formed from ATP\(^{32}\) in the presence of cabbage phosphatidic acid (Table V). The addition of \(\alpha\)-glycerocephosphate did not appreciably affect the incorporation into phosphatidic acid of P\(^{32}\) from ATP\(^{32}\) either in the absence or presence of added substrate. These results indicate that the mechanism for the synthesis of phosphatidic acid...
Phosphatidic Acid Phosphatase in Soluble Extracts—When cabbage phosphatidic acid was added to soluble extracts of deoxycholate-treated brain microsomes, orthophosphate was liberated (Table VI). The extracts showed some specificity toward cabbage phosphatidic acid; distearoyl phosphatidic acid was only feebly hydrolyzed. α-Glycerophosphate was hydrolyzed about one half as effectively as cabbage phosphatidic acid, indicating that the liberation of orthophosphate from cabbage phosphatidic acid could not proceed entirely via α-glycerophosphate as an intermediate. β-Glycerophosphate was hydrolyzed more slowly than α-glycerophosphate (Table VI).

The activity of the cabbage phosphatidic acid phosphatase was increased about 100 per cent upon addition of magnesium ions. However, there was no absolute requirement for magnesium ions. Smith et al. (22) have described an enzyme present in particular material from rat heart, kidney, brain, liver, and skeletal muscle, and from chicken liver, which catalyzes the liberation of orthophosphate from phosphatidic acid prepared from egg lecithin; this enzyme was inhibited by magnesium ions.

A concentration of cabbage phosphatidic acid of 0.001 M was a near maximal concentration for the activity of phosphatidic acid phosphatase. The activity was inhibited about 80 per cent in the presence of 1.5 × 10⁻³ M p-chloromercuribenzoate; this indicates that the enzyme probably has active sulfhydryl groups (Table VI).

The activity of phosphatidic acid phosphatase provided diglyceride from cabbage phosphatidic acid for phosphatidic acid synthesis in soluble extracts in the presence of the ATP₃₂-generating system. In addition to this, part of the increase in radioactivity of phosphatidic acid after incubation with cabbage phosphatidic acid was due to a dilution effect of the added carrier on the hydrolysis of the newly formed P₃₂-labeled phosphatidic acid. As compared with extracts which had been incubated with 0.0004 M diglyceride (which is the maximal amount which might be expected to be formed from 0.002 M cabbage phosphatidic acid, Table VI), an average of 150 per cent more P₃₂-labeled phosphatidic acid was recovered from the extracts which had been incubated with 0.002 M cabbage phosphatic acid. This action as a carrier diluent can explain the observation that, when the phosphatidic acid was formed from α-glycerophosphate, the addition of cabbage phosphatidic acid increased the amount of P₃₂-labeled phosphatidic acid recovered by about 90 per cent (Table V).

In the presence of optimal amounts of diglyceride, cabbage phosphatidic acid produced some inhibition of phosphatidic acid synthesis. Discussion

Diglyceride and ATP appear to be the only substrates required for the synthesis of phosphatidic acid in soluble dialyzed extracts of deoxycholate-treated brain microsomes. This would suggest the following reaction for the synthesis of phosphatidic acid under these conditions:

\[
\text{Diglyceride} + ATP \rightarrow \text{Phosphatidic Acid}
\]

The following table shows the incorporation of glycerol-¹⁴C and ATP into phosphatidic acid in soluble extracts.

**Table V**

Comparison of incorporation of glycerol-¹⁴C and ATP into phosphatidic acid in soluble extracts.

<table>
<thead>
<tr>
<th>Other additions</th>
<th>P₃₂-labeled phosphatidic acid</th>
<th>ATP</th>
<th>α-Glycerophosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmpoles</td>
<td>mmpoles</td>
<td>mmpoles</td>
</tr>
<tr>
<td>None</td>
<td>0.0018</td>
<td>0.72</td>
<td>0.72</td>
</tr>
<tr>
<td>Cabbage phosphatidic acid</td>
<td>0.0029</td>
<td>6.20</td>
<td>6.20</td>
</tr>
<tr>
<td>Cabbage phosphatidic acid + α-glycerophosphate</td>
<td>0.0012</td>
<td>5.05</td>
<td>1.88</td>
</tr>
<tr>
<td>Linoleic acid + linolenic acid</td>
<td>1.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linoleic acid + linolenic acid + α-glycerophosphate</td>
<td>1.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linoleic acid + linolenic acid + glycerol</td>
<td>1.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Glycero-P</td>
<td>0.0023</td>
<td>1.53</td>
<td>1.53</td>
</tr>
<tr>
<td>α-Glycero-P (0.005 M)</td>
<td>0.72</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>α Glycero P (0.01 M)</td>
<td>0.75</td>
<td>0.75</td>
<td></td>
</tr>
</tbody>
</table>

*All 840 mmpoles per vessel (0.002 M), except as otherwise indicated.
†Final concentration, 0.002 M.
‡p-Chloromercuribenzoate, 1.5 × 10⁻³ M.

**Table VI**

Phosphatidic acid phosphatase activity in soluble extracts of deoxycholate-treated brain microsomes.

Aliquots (0.2 ml.) of a soluble extract were added to 0.2 ml. of 0.4 M potassium glycglycine, pH 6.5. Incubations were carried out for 1 hour at 37° with shaking. The amount of orthophosphate liberated is that obtained from an aliquot which represented one fifteenth of the total yield of soluble extract from the microsomes of the cerebral hemispheres of one guinea pig. Incubations and estimations were carried out in duplicate. All figures are corrected for the amount of orthophosphate liberated when enzyme and substrate were incubated in separate vessels. With the exception of L-α(distearoyl)phosphatidic acid, a negligible amount of orthophosphate was found after incubation of the substrates without enzyme. The amount of orthophosphate found after incubation of the enzyme preparation without substrate was about 100 mmpoles in the undialyzed preparations and about 20 mmpoles after dialysis.

<table>
<thead>
<tr>
<th>Substrate*</th>
<th>Other conditions</th>
<th>Orthophosphate liberated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mmpoles</td>
</tr>
<tr>
<td>Undialyzed enzyme preparation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cabbage phosphatidic acid</td>
<td>Mg⁺⁺ omitted</td>
<td>95</td>
</tr>
<tr>
<td>Cabbage phosphatidic acid</td>
<td>MgSO₄ added</td>
<td>167</td>
</tr>
<tr>
<td>α-Glycerophosphate</td>
<td>MgSO₄ added</td>
<td>23</td>
</tr>
<tr>
<td>β-Glycerophosphate</td>
<td>MgSO₄ added</td>
<td>40</td>
</tr>
<tr>
<td>Dialyzed enzyme preparation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cabbage phosphatidic acid</td>
<td>Mg⁺⁺ omitted</td>
<td>88</td>
</tr>
<tr>
<td>Cabbage phosphatidic acid</td>
<td>MgSO₄ added</td>
<td>133</td>
</tr>
<tr>
<td>Cabbage phosphatidic acid + CMBI</td>
<td>MgCl₂ added</td>
<td>146</td>
</tr>
<tr>
<td>Cabbage phosphatidic acid (320 mmpoles)</td>
<td>MgCl₂ added</td>
<td>120</td>
</tr>
</tbody>
</table>

*All 840 mmpoles per vessel (0.002 M), except as otherwise indicated.
†Final concentration, 0.002 M.
‡p-Chloromercuribenzoate, 1.5 × 10⁻³ M.
Diglyceride + ATP $\rightarrow$ diglyceride kinase

phosphatidic acid + ADP

Since magnesium ions are required for the ATP$^{a2}$-generating system used here, it was not possible to test whether divalent ions are a requirement for the diglyceride kinase system.

It has been shown elsewhere that brain phosphatidic acid is chromatographically indistinguishable from cabbage phosphatidic acid; it is chromatographically distinguishable from dis- 
tearyl phosphatidic acid but becomes indistinguishable after hydrogenation (21). That both brain phosphatidic acid phosphatase and brain diglyceride kinase show the greatest activity towards cabbage phosphatidic acid or the diglyceride from it is further evidence that one or more of the diglycerides of cabbage phosphatidic acid must be closely related to the diglycerides of brain phosphatidic acid, more closely related, in fact, than are the diglycerides from brain lecithin.

**SUMMARY**

Phosphatidic acid is synthesized from diglyceride and adenosine triphosphate-P$^{32}$ in soluble extracts of deoxycholate-treated brain microsomes. The most effective substrate is diglyceride from cabbage phosphatidic acid; diglyceride from brain lecithin, and 1-palmitoyl-2-oleyl diglyceride are much less effective. Glycero-phosphate is not an intermediate in this reaction. Diglyceride kinase activity is present in the intact microsomes, but added substrate is not utilized unless surface-active agents such as deoxycholate are added. The synthesis of phosphatidic acid by this pathway in brain microsomes is greatly increased after treatment of the microsomes with suitable concentrations of deoxycholate; however, very low and very high concentrations of deoxycholate inhibit the synthesis.

The soluble extracts of deoxycholate-treated microsomes also contain an enzyme which catalyzes the liberation of orthophosphate from cabbage phosphatidic acid. Addition of magnesium ions increases the activity of this phosphatase. The combined action of the phosphatidic acid phosphatase and the diglyceride kinase in these extracts catalyzes the exchange of phosphate in cabbage phosphatidic acid.

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**REFERENCES**

The Synthesis of Phosphatidic Acid from Diglyceride and Adenosine Triphosphate in Extracts of Brain Microsomes
Mabel R. Hokin and Lowell E. Hokin


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