Deacylation of Phospholipids and Acylation and Deacylation of Lysophospholipids Containing Ethanolamine, Choline, and β-Methylcholine by Microsomes from Housefly Larvae*

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ABSTRACT: A microsomal preparation (40,000–90,000*g* sediment) obtained from housefly larvae converts exogenous phosphatidylcholine, phosphatidylethanolamine, and phosphatidyl-β-methylcholine into the respective glycerophosphorylbases. The pH optimum is near 8.0, and the overall conversion is stimulated by 2.5 mM lauryl sulfate and is inhibited by Ca^{2+}. Ca^{2+} primarily inhibits production of lysophospholipids. The combination of 1 mM $HgCl_2$ and 2.5 mM lauryl sulfate or 5 mM deoxycholate greatly stimulates the rate of production of the lysophospholipids but inhibits glycerophosphoryl base formation from diacyl glycerophosphatides. In contrast, the combination of low concentrations of lauryl sulfate or deoxycholate and $HgCl_2$ inhibits lysophospholipid formation. The 2-acyl-*sn*-glycero-3-phosphoryl bases accumulate in the reaction media and are also deacylated by larval microsomes. Ca^{2+} and lauryl sulfate stimulate cleavage of fatty acids from 1-acyl-*sn*-glycero-3-phosphorylethanolamine and 2-acyl-*sn*-glycero-3-phosphorylethanolamine. The data are consistent with the occurrence of phospholipase A_1 (EC 3.1.1.4) and at least one lysophospholipase (EC 3.1.1.5) activity in larval microsomes. The microsomes also contain acyl transferases which convert 1-acylglycero-3-phosphoryl derivatives of choline, β-methylcholine, and ethanolamine into the respective diacyl glycerophosphatides in the presence of acylcoenzyme A.

The rate of fatty acid cleavage from 1-acyl-*sn*-glycero-3-phosphoryl bases is greater than the rate of acylation; however, the apparent K_m of 6×10^{-4} M for deacylation of 1-acyl-*sn*-glycero-3-phosphorylcholine is 20-fold greater than the apparent K_m for acylation, indicating that at low lysophospholipid concentrations found *in vivo*, acylation of the lysophospholipids could be the preferred reaction. Rates as high as 40 mμmoles/min per mg of protein for formation of 2-acyl-*sn*-glycero-3-phosphorylethanolamine and formation of 2-acyl-*sn*-glycero-3-phosphorylcholine from the respective diacyl glycerophosphatides were obtained. The microsomes can be used for making preparative amounts of 2-acyl-*sn*-glycero-3-phosphoryl bases.

It is well established that some dipterans can form unusual phospholipids (Bieber and Newburgh, 1963a,b; Bridges *et al.*, 1965; Bridges and Ricketts, 1967; Hodgson *et al.*, 1969). One of the unusual phosphatides, phosphatidyl-β-methylcholine, is derived from a natural product, carnitine; however, little is known concerning the metabolic fate of this phosphatide or insect phospholipids in general. A few investigations of insect phospholipases, that are not constituents of venoms, have been reported. Khan and Hodgson (1967) reported the presence of phospholipase A- and B-type activity in the housefly, *Musca domestica*, and in the blowfly, *Phormia regina*, and Bieber *et al.* (1968) and Hildenbrandt *et al.* (1969) reported that a microsomal fraction obtained from housefly larvae rapidly converted phospholipids into the respective glycerophosphoryl bases. Phospholipases occur in Culex larvae (Rao and Subrahmanyam, 1969a,b), and phosphatidate hydrolase is present in Cecropia fat body (Hirano and Gilbert, 1967).

In this paper, we report some properties of phospholipase A_1 (EC 3.1.1.4), lysophospholipase (EC 3.1.1.5), and acyl transferase that are present in a 40,000–90,000*g* microsome-containing fraction obtained from housefly larvae. Diacyl glycerophosphatides and 1-acyl-*sn*-glycero-3-phosphatides containing choline, ethanolamine, and β-methylcholine were used as substrates and conditions for formation of the respective 2-acyl-*sn*-glycero-3-phosphoryl bases are described.

Experimental Procedures

Isolation of the Microsome-Containing Fraction. Housefly larvae were reared aseptically by the method of Monroe (1962) and third instar (mature, 3-days old) larvae were used for all experiments. Larvae were suspended in distilled water and collected in a fine-mesh tea strainer. Washed larvae (15 g) in 30 ml of buffer were homogenized for 30 sec at 4° with a micro Waring Blendor. The buffer (pH 7.2) contained 0.05 M Tris-HCl, 0.1 M sucrose, and 0.001 M EDTA. The homogenate was filtered through eight layers of cheesecloth and nuclei and debris were removed by centrifugation at 500*g* for 10 min. Mitochondria and lysosomes were removed by centrifugation at 10,000*g* and 40,000*g*, respectively, at 4° for 10 min. The microsome-containing fraction used in the experiments was obtained by centrifuging the 40,000*g* supernatant at 92,000*g* for 1 hr. The supernatant was decanted and the pellet was rinsed with 5 ml of 0.05 M Tris-HCl buffer (pH 7.2). The pellet was suspended in the buffer with the aid of a glass rod and recentrifuged at 92,000*g* for 1 hr. The washed pellet was sus-

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pended in 0.05 M Tris-HCl buffer (pH 7.2) and the volume was made to 5 ml.

Preparation of ^{32}P-Labeled Phospholipids. Housefly larvae were raised axenically on a diet containing 30–50 μCi of carrier-free [^{32}P]H_3PO_4/flask, and the ^{32}P-labeled lipids were extracted therefrom by the method of Folch *et al.* (1957) and purified by silicic acid chromatography as described elsewhere (Bieber *et al.*, 1969). Phosphatidyl-β-methylcholine was obtained as described previously (Bieber *et al.*, 1969). Thin-layer chromatography of ^{32}P-labeled phosphatidylethanolamine, phosphatidylcholine, and phosphatidyl-β-methylcholine demonstrated the absence of the respective lysophosphatides; impurities present in amounts <1–3% of the total lipid phosphorus would not have been detected. Preparations of ^{32}P-labeled phosphatidyl-β-methylcholine contained approximately 5% ^{32}P-labeled phosphatidylcholine, as reported previously (Bieber *et al.*, 1969). The lipids were stored in chloroform at −20°. ^{32}P-Labeled 1-acyl lysophospholipids were prepared from purified ^{32}P-labeled diacyl glycerophosphatides by sonicating the phospholipids in 0.05 M Tris-HCl (pH 7.4) and then adding 10 mM $CaCl_2$, 0.1% Triton X-100, and *Crotalus adamanteus* venom. ^{32}P-Labeled 2-acyl lysophospholipids were prepared by reacting ^{32}P-labeled phospholipids with larval microsomes in the presence of 1 mM $HgCl_2$ and 2.5 mM lauryl sulfate. The reactions were terminated by adding five volumes of chloroform–methanol (2:1, v/v) and an equal volume of water was added. After mixing and separating the layers, the chloroform layer was evaporated to dryness and the residue was redissolved in chloroform. The lysophospholipids were separated from diacyl glycerophosphatides by column chromatography on silicic acid or by preparative thin-layer chromatography using silica gel G plates. The solvent system was chloroform–methanol–H_2O (65:35:4).

Assays. Lipids were dispersed in 0.05 M Tris-HCl buffer (pH 7.2) by sonication for 30 sec at 50 W using a Branson Sonifier, Model W185D. The final volume was made such that 1 μmole of phospholipid phosphorus was present in 0.1 ml. Unless otherwise indicated, the reaction mixture contained: 40 mM Tris-HCl buffer (pH 8.0), 1 mM substrate, and 0.2 ml of enzyme in a final volume of 1 ml. Controls did not contain the enzyme and each assay was done in duplicate with one control. Incubations were performed at 30° in a Dubnoff metabolic shaker. The reaction was terminated by addition of 3.0 ml of chloroform–methanol (2:1). Then 0.1 ml of 0.2 M $MgCl_2$ was added and the samples were mixed using a Vortex mixer. The water and chloroform layers were separated by centrifugation and the water layers were removed with a Pasteur pipet. The chloroform layer was washed with 1 ml of 0.02 M $MgCl_2$ and the water extracts were combined. The water extract was washed with 1 ml of chloroform, and the chloroform was added to the lipid extract. The water extract was made to 3.0 ml and 0.3 ml was counted in 10.0 ml of water utilizing Cerenkov radiation, as described by Haviland and Bieber (1969).[1] The amount of water-soluble material was calculated from the specific activity of the substrate. The lipid extracts were evaporated and dissolved in a small volume of chloroform. Aliquots were applied on silica gel F-254 (Brinkman) plates and developed in $CHCl_3$–MeOH–H_2O (65:35:4).

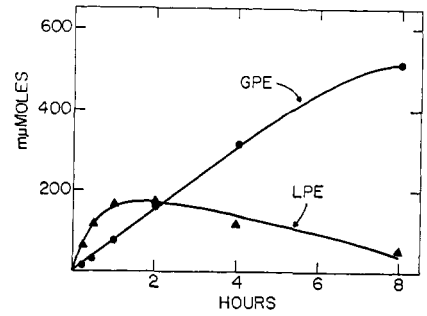


FIGURE 1: Deacylation of phosphatidylethanolamine by housefly larvae microsomes. The incubation contained in 10 ml, 4.0 ml of 0.1 M Tris-HCl buffer (pH 8.0), 10 μmoles of substrate, and 2 ml of enzyme preparation. Aliquots were taken at the various times and pipetted into 3 ml of a chloroform–methanol mixture (2:1). The extraction procedure was the same as described in the Methods. (▲—▲) Lysophosphatidylethanolamine = LPE; (●—●) Glycerophosphorylethanolamine = GPE.

Phospholipids were visualized with iodine and marked. After removal of the iodine a 16-cm^2 area was scraped into scintillation vials and counted by Cerenkov radiation[2] (see Haviland and Bieber, 1969), and the per cent of lysophospholipid in the reaction mixture was determined.

The water-extractable products were identified by paper chromatography using the solvents picric acid–*t*-butyl alcohol–H_2O (4 g:80:20, wt/v/v) or methanol–concentrated ammonium hydroxide–water (12:2:3). For the latter solvent, water extracts were passed through a small Dowex 50 (H^+) column to remove the magnesium ions. The phosphate esters were detected using a molybdate–perchloric acid spray (Bandurski and Axelrod, 1951) and amino-containing compounds were detected with ninhydrin.

Results

Deacylation of Exogenous Phosphatidylcholine, Phosphatidylethanolamine, and Phosphatidyl-β-methylcholine by Housefly Microsomes. When ^{32}P-labeled phosphatidylethanolamine, phosphatidylcholine, or phosphatidyl-β-methylcholine were incubated with the 40,000–90,000*g* sediment from housefly larvae, both lysophospholipids and glycerophosphoryl bases were formed, as reported previously (Bieber *et al.*, 1968; Hildenbrandt *et al.*, 1969). The rate of phosphatidylethanolamine deacylation was relatively constant for up to 4 hr, as shown in Figure 1. In some experiments, production of both lysophosphatidylethanolamine and glycerophosphorylethanolamine was linear for more than 1 hr. Lysophosphatidylethanolamine attained an apparent constant level and then declined. The pH optimum for the overall conversion of the diacyl glycerophosphatides into glycerophosphoryl bases was 8.0. A similar pH optimum was observed for the formation of lysophospholipids, as is shown in Figure 2.

Effects of Cations and Detergents on Deacylation of Diacylglycerophosphatides by Housefly Microsomes. Initial experiments with phosphatidylcholine and phosphatidyl-β-methylcholine as substrates were performed in the presence of Ca^{2+},

[1] Details of this procedure will be furnished on request. The principle of Cerenkov radiation is described in Packard Technical Bulletin No. 16, Jan 1966.

[2] The area was constant at 4 × 4 cm because the absolute efficiency of counting dry silicic acid is dependent on the mass of the media.

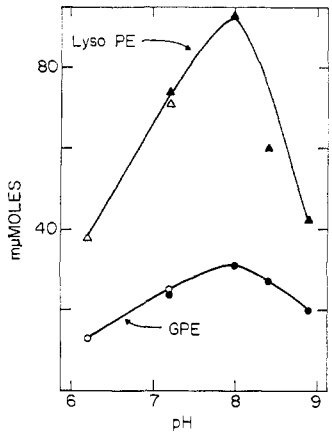


FIGURE 2: Effect of pH on hydrolysis of phosphatidylethanolamine by housefly larvae microsomes. The samples were incubated for 30 min at 30° with 0.56 mg of protein. The incubation mixture in 1.0 ml contained 0.4 ml of 0.1 M Tris-HCl or 0.1 M imidazole-HCl buffer and 1 μmole of substrate. ○ and △ represent imidazole buffer, and ● and ▲ represent Tris buffer.

and production of lysophospholipids and glycerophosphoryl bases was obtained; however, when Ca^{2+} concentrations greater than 1 mM were used, inhibition of glycerophosphoryl base formation was consistently observed. Formation of lysophosphatidylethanolamine and glycerophosphorylethanolamine was inhibited by 1 and 2.5 mM $CaCl_2$ (see Table I) and, as shown in Table II, $CaCl_2$ (>1 mM) also inhibited the production of glycerophosphorylcholine. When Hg^{2+} was added to inhibit potential transacylations, formation of lysophosphatidylcholine and lysophosphatidyl-β-methylcholine, and the respective glycerophosphoryl bases was inhibited by 1 mM $HgCl_2$. In contrast, lysophosphatidylethanolamine and glycerophosphorylethanolamine formation was not inhibited by 1 mM $HgCl_2$ (see Tables I and II).

The presence of 2.5 mM lauryl sulfate stimulated production of lysophospholipids approximately fourfold and also stimulated glycerophosphoryl base formation (see Table II). The combination of 1 mM $HgCl_2$ and 2.5 mM lauryl sulfate stimulated formation of lysophosphatides but inhibited formation of glycerophosphoryl bases. In contrast, the combination of lauryl sulfate and $HgCl_2$ inhibited lysophospholipid production when the concentration of lauryl sulfate was 1 mM or less, as shown in Figure 3A. Similar results were obtained when lauryl sulfate was replaced by deoxycholate except that higher concentrations of deoxycholate were required before stimulation of lysophospholipid formation occurred; see Figure 3B in which phosphatidylethanolamine was the substrate. Phosphatidylcholine and phosphatidyl-β-methylcholine were not used with deoxycholate.

Deacylation of phosphatidylcholine was faster than deacylation of phosphatidyl-β-methylcholine in all experiments with the exception that the lyso derivatives were produced in approximately equal amounts in the presence of 2.5 mM lauryl sulfate. Phosphatidylethanolamine was deacylated as fast as phosphatidylcholine, but not as rapidly as 1-acyl-*sn*-glycero-3-phosphorylethanolamine (compare Figure 3 and Tables II and III).

Acylation and Deacylation of Lysophospholipids by Microsomes. When the microsome-containing fraction was incubated with ^{32}P-labeled 1-acyl-*sn*-glycero-3-phosphoryl derivatives of choline, ethanolamine, or β-methylcholine in the presence of palmitoylcoenzyme A or a mixture of ATP, coenzyme A, oleate, and palmitate, the respective diacyl glycerophosphatides were formed. The pH optimum of these acylations was

TABLE I: Effect of Mercuric and Calcium Ions on Degradation of Phosphatidylethanolamine by Housefly Larvae Microsomes.[a]

Additions	Rel Act.	
	Lyso-PE	GPE
None	100	100
$HgCl_2$, 1 mM	186	108
$CaCl_2$, 0.5 mM	102	74
$CaCl_2$, 1.0 mM	34	31
$CaCl_2$, 2.5 mM	9	8

[a] Each reaction in a final volume of 1 ml contained 40 mM Tris-HCl buffer (pH 8.0), 1 μmole of substrate, metal ions as indicated, and 0.73 mg of protein. Incubation was for 1 hr at 30°. Lyso-PE (101 mμmoles) and GPE (98 mμmoles) were formed in the control. Lyso-PE, lysophosphatidylethanolamine; GPE, glycero-3-phosphorylethanolamine.

TABLE II: Effect of Cations and Lauryl Sulfate on the Deacylation of Phosphatidylcholine and Phosphatidyl-β-methylcholine by Larval Microsomes.[a]

Additions (mM)	mμmoles/mg of Protein per 15 min			
	PC as Substrate		PMC as Substrate	
	Lyso-PC	GPC	Lyso-PMC	GPMC
None	74	17	34	8.6
$HgCl_2$ (1)	36	10	11	3.0
Lauryl sulfate (2.5)	336	97	325	46.0
$HgCl_2$ (1) + lauryl sulfate (2.5)	559	39	413	20.0
$HgCl_2$ + lauryl sulfate (2.5) + $CaCl_2$ (1)	280	20	196	10.0
Lauryl sulfate (2.5) + $CaCl_2$ (1)	235	53	147	22.0
$CaCl_2$ (0.5)	45	19	22	8.0
$CaCl_2$ (1)	37	10	17	7.0
$CaCl_2$ (2.5)	28	7	14	9.0

[a] Substrate (1 μmole) in 1 ml was used and incubations and assays were performed as described for Figures 1 and 2. PC, phosphatidylcholine; PMC, phosphatidyl-β-methylcholine; Lyso-PC, lysophosphatidylcholine; Lyso-PMC, lysophosphatidyl-β-methylcholine; GPC, glycero-3-phosphorylcholine; GPMC, glycero-3-phosphoryl-β-methylcholine.

TABLE III: Deacylation of 1-Acyl-*sn*-glycero-3,-phosphorylethanolamine and 2-Acyl-*sn*-glycero-3-phosphorylethanolamine by Housefly Larval Microsomes.[a]

Additions (mM)	mμmoles/15 min per mg of Protein		
	1-Acyl-GPE		2-Acyl-GPE
	GPE	PE	GPE
None	461	8	515
$HgCl_2$ (1)	765	14	715
Lauryl sulfate (2.5)	640	4	525
$HgCl_2$ + lauryl sulfate	837	6	580
$CaCl_2$ (0.5)	748	11	
$CaCl_2$ (1.0)	627	8	
$CaCl_2$ (2.5)	1046	7	
$CaCl_2$ (5.0)	1069	6	784

[a] For 1-acyl-GPE as substrate, the reaction mixture contained in 1 ml: 0.68 mg of protein, 1 mM substrate, 40 mM Tris buffer (pH 8.0), and 0.2 ml of enzyme solution. Incubations and assays were performed as described in Methods. Similar conditions were used when 2-acyl-GPE was the substrate except that the volume was reduced to 0.5 ml and 0.34 mg of protein was used. GPE, glycerophosphorylethanolamine; PE, phosphatidylethanolamine; 1-Acyl-GPE, 1-acyl-*sn*-glycero-3-phosphorylethanolamine; 2-Acyl-GPE-GPE, 2-acyl-*sn*-glycero-3-phosphorylethanolamine.

near 6.5. The rate of deacylation by phospholipases was greater than the rate of acylation by the acyl transferases, even at pH 6.5 and also at pH 7.4, as shown in Figure 4 in which lysolecithin was the substrate. Similar results were obtained using lysophosphatidylethanolamine as substrate.

$CaCl_2$ at 5×10^{-4}–10^{-2} M Ca^{2+} stimulated production of glycerophosphoryl derivatives of choline, β-methylcholine, and ethanolamine when 1-acyl lysophospholipids were substrates. Ca^{2+} stimulated deacylation of both 1-acyl and 2-acyl lysophospholipids (see Table III). Concentrations of less than 5 mM lauryl sulfate also stimulated production of the glycerophosphoryl bases from 1-acyl lysophospholipids containing choline, β-methylcholine, or ethanolamine, and as shown in Table III, $HgCl_2$ and $HgCl_2$ plus 2.5 mM lauryl sulfate stimulated deacylation of both 1-acyl-*sn*-glycero-3-phosphorylethanolamine and 2-acyl-*sn*-glycero-3-phosphorylethanolamine.

Apparent K_m's for some of the lysophospholipids using a double-reciprocal plot were estimated. The acyl transferase had an apparent K_m of 3×10^{-5} M for 1-acyl-*sn*-glycero-3-phosphorylcholine and 2×10^{-5} M for palmitoylcoenzyme A. The apparent K_m's for deacylation of 1-acyl-*sn*-glycero-3-phosphoryl derivatives of choline and ethanolamine were both 6×10^{-4} M.

Position of the Acyl Residue of Lysophosphatidylethanolamine Formed by Larval Microsomes. When larval microsomes were incubated with phosphatidylethanolamine that contained ^{14}C in the acyl group on position number one, in the presence of $HgCl_2$ and 2.5 mM lauryl sulfate, ^{14}C was not detected in the isolated lysophosphatidylethanolamine demonstrating removal of the 1-acyl residue from phosphatidylethanolamine (see Table IV). When the same ^{14}C-labeled phosphatidylethanolamine was incubated with *Crotalus adamanteus* venom, the data within experimental error demonstrated that the ^{14}C-labeled acyl residue was not removed; it was with the lysophosphatidylethanolamine. When larval microsomes were incubated with ^{14}C-labeled phosphatidylethanolamine in the absence of $HgCl_2$ and lauryl sulfate, both glycerophosphorylethanolamine and lysophosphatidylethanolamine were formed. If one accounts for the two fatty acids produced per glycerophosphorylethanolamine formed and the 5% lysophosphatidylethanolamine formed nonenzymatically, then most, if not all, of the lysophosphatidylethanolamine formed by the microsome preparation had the acyl residue on the number two carbon of glycerol.

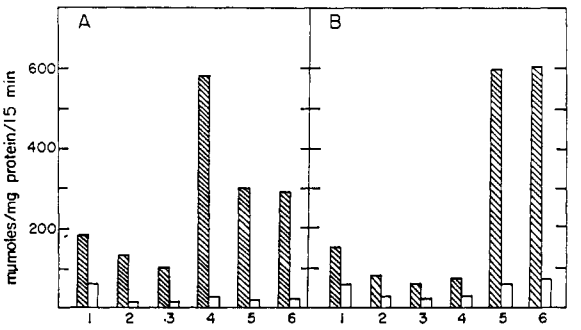


FIGURE 3: Effect of lauryl sulfate and deoxycholate on degradation of phosphatidylethanolamine by larval microsomes. The incubation mixture contained in 1.0 ml, 0.4 ml of 0.1 M Tris-HCl buffer (pH 8.0), 1 mM $HgCl_2$, 1 μmole of substrate, 0.73 mg of protein, and lauryl sulfate or deoxycholate. (1) Mercuric chloride (1 mM), (2) $HgCl_2$ (1 mM) + detergent (0.5 mM), (3) $HgCl_2$ (1 mM) + detergent (1.0 mM), (4) $HgCl_2$, (1 mM) + detergent (2.5 mM), (5) $HgCl_2$ (1 mM) + detergent (5.0 mM), and (6) $HgCl_2$ (1 mM) + detergent (10.0 mM). The detergent used in A was lauryl sulfate, and the detergent used in B was deoxycholate. The open areas represent glycerophosphorylethanolamine and the hatched areas represent lysophosphatidylethanolamine.

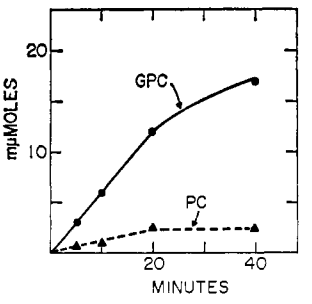


FIGURE 4: Time course of acylation and deacylation of lysolecithin by housefly larvae microsomes. The incubation mixtures contained 0.15 μmole each of lysolecithin and palmitoyl-CoA, 0.8 mg of microsomal protein, and 0.1 M Tris-HCl (pH 7.4) to bring the mixture to 1.5 ml. Lecithin formed (Δ—Δ); water-soluble counts formed (○—○). GPC = glycerophosphorylcholine; PC = phosphatidylcholine. The reaction was terminated and analyzed for lecithin, lysolecithin, and glycerophosphorylcholine, as described in the Methods.

TABLE IV: Position of the Acyl Residue on Accumulated Lysophosphatidylethanolamine.[a]

Incubation Conditions	Sample	Distribution of ^{14}C (%)			% of Init PE Converted into GPE	Position of the Acyl Residue on the Isolated Lyso-PE	% PE Converted into Lyso-PE
		Lyso-PE	Fatty Acids	PE			
Larval microsomes +	1	0	59	41			
2.5 mM lauryl sulfate	2	0	49	51	1	All 2-acyllyso-PE	55
+ 1 mM $HgCl_2$	Control	4	1	95			
C. adamanteus venom	1	90	0	10			
+ 10 mM $CaCl_2$	2	90	3	7	1	>95% 1-acyllyso-PE	90
	Control	5	2	93			
Larval microsomes	1	7	40	53		Mainly 2-acyllyso-PE;	
No additions	2	8	38	54	11	some 1-acyllyso-PE	20
	Control	5	2	93		could be present.	

[a] Phosphatidylethanolamine containing ^{32}P or ^{14}C was prepared by injecting rats with [^{32}P]P_i or [^{14}C]palmitate. Lipids were extracted from the liver by the method of Folch *et al.* (1957) and purified as described in the Methods; ^{14}C- and ^{32}P-labeled phosphatidylethanolamine was mixed in a 4:1 molar ratio. The reaction mixtures were identical with those described in the Methods. The *C. adamanteus* venom assay was done at pH 7.2. The reaction products were partitioned as described in the Methods except that after thin-layer chromatography, the spots were scraped from the plate, pulverized, and counted in toluene containing 4 g of 2,5-diphenyloxazole plus 100 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene/l. and 4% Cab-O-Sil (w/v). Channel settings were the same as described by Van den Bosch and Van Deenen (1966). PE, phosphatidylethanolamine; Lyso-PE, lysophosphatidylethanolamine; GPE, glycero-3-phosphorylethanolamine. The yield of lyso-PE was 55% in the presence of lauryl sulfate.

The larval microsome preparations contained lipase activity —determined by H^+ release in the presence of olive oil using a pH-Stat—and also contained some acid phosphatase activity indicative of lysosomes.

Discussion

The experiments reported herein using exogenous substrates clearly demonstrate that a microsome-containing fraction from housefly larvae has enzymatic activity for removal of both fatty acids from the two most prevalent phospholipids of the housefly; namely, phosphatidylcholine and phosphatidylethanolamine. This fraction also deacylates phosphatidyl-β-methylcholine, a phospholipid that apparently can substitute for much of the phosphatidylcholine in housefly larvae. Presumably, these phospholipases also deacylate other uncommon and unnatural phospholipids that can be formed by housefly larvae.

Formation of lysophospholipids from phosphatidylcholine, phosphatidyl-β-methylcholine, and phosphatidylethanolamine, as well as production of the respective glycerophosphoryl bases, was inhibited by millimolar concentrations of $CaCl_2$. Inhibition of glycerophosphoryl base production by Ca^{2+} is partially, if not entirely, due to a reduction in the amount of precursors; namely, the lysophospholipids. This is demonstrated by the data given in Table II where the combination of lauryl sulfate and Ca^{2+} caused a threefold increase in glycerophosphorylcholine, but this amount of glycerophosphorylcholine was 60% that of the sample that contained lauryl sulfate without Ca^{2+}. Deacylation of 1-acyl-*sn*-glycero-3-phosphoryl bases and 2-acyl-*sn*-glycero-3-phosphoryl bases in the absence of exogenous diacyl glycerophosphatides was stimulated by $CaCl_2$.

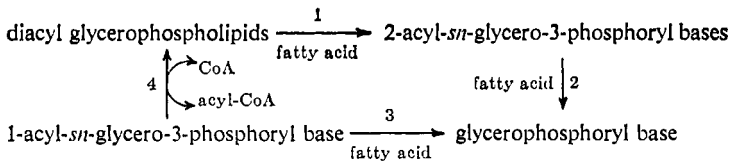
The effect of lauryl sulfate and deoxycholate is variable and complex. These micelle formers stimulated lysophospholipid and glycerophosphoryl base production from diacyl glycerophosphatides at optimum concentrations, but were inhibitory at low concentrations. Stimulation of glycerophosphoryl base formation by the detergents was probably caused by an increase in the amount of precursors; namely, the lysophospholipids. The variable detergent effects might be caused by changes in the type of micelles that are formed at different detergent concentrations. Such changes could affect the accessibility of substrates.

The accumulation of lysophospholipids in the presence of 1 mM $HgCl_2$ and 2.5 mM lauryl sulfate provided a convenient source of lysophospholipid for subsequent experimentation. The data demonstrate that the acyl residue of the accumulated lysophospholipid is mainly, if not entirely, located on the number two carbon of the glycerol moiety. The accumulation of lysophospholipids appears to be due to a stimulation of lysophospholipid formation and to an inhibition of the lysophospholipase that removes the acyl residue from the number two position. The inhibition of lysophospholipase activity may be caused by the diacyl glycerophosphatides; Van den Bosch *et al.* (1968) reported such an inhibition of rat liver lysophospholipase.

The data presented herein demonstrate that housefly larvae have particle-bound enzymes that catalyze the reactions seen in Scheme I.

Van Deenen and De Haas (1963) have shown that phospholipase A_2 from *C. adamanteus* catalyzes cleavage of the acyl residue from the number two position of diacyl and monoacyl glycerophosphatides; however, the fact that Ca^{2+} inhibits reaction 1 but stimulates reaction 3 indicates two different enzymes; namely, phospholipase A_2 and lysophospholipase.

SCHEME I



The conclusion that 2-acyl-*sn*-glycero-3-phosphoryl bases are intermediates in the conversion of diacyl glycerophosphatides into the glycerophosphoryl bases by larval microsomes appears warranted since 2-acyl-*sn*-glycerophosphoryl bases are formed and are also deacylated by larval microsomes. Similar results have been found in other microsomal systems.

The enzymatic activities reported herein are primarily, if not entirely, microsomal and not due to mitochondria or lysosomes. Succinic dehydrogenase activity was not detected in the preparations, indicating absence of mitochondria, but the preparations contained some acid phosphatase indicative of lysosomes. The pH optimum near 8.0 for the production of lysophosphatides and the respective glycerophosphoryl bases is not indicative of lysosomes. Deacylation of phospholipids was as high as 40–50 mμmoles/min per mg of protein and the deacylation rate for 1-acyl-*sn*-glycero-3-phosphorylethanolamine was even greater. Such high activities are much greater than would be expected for lysosomes 3–4 pH units above their optimum.

The data demonstrate that larval microsomes in the presence of acylcoenzyme A can convert lysophospholipids into diacyl glycerophospholipids. The microsomal location of this activity is consistent with the subcellular distribution of acyl transferases reported by Eibl and coworkers (1969). Although the acylation of lysophospholipids by larval microsomes is slower than deacylation, acylation may be the preferred reaction at the low lysophospholipid concentrations usually present *in vivo*. This contention is supported by the fact that the approximate K_m's for 1-acyl-*sn*-glycerophosphatides containing ethanolamine and choline are 20-fold greater for deacylation than for acylation. Thus, normally, microsomes may reacylate lysophospholipids and the lysophospholipases could prevent accumulation of deleterious quantities of lysophospholipids. The high capacity for glycerophosphoryl base production could be related to the fact that the larvae were approaching the prepupal stage of development, a stage in which considerable hydrolytic activity occurs.

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