Hydrolysis of phospholipids by a lysosomal enzyme

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ABSTRACT The phospholipid-hydrolyzing activity of rat liver lysosomes has been studied. These lysosomes contain a phospholipase that cleaves both fatty acid ester linkages of lecithin and of phosphatidyl ethanolamine and releases free fatty acids from both positional isomers of lysolecithin. The enzyme does not require calcium for maximum activity, and is inhibited by diethyl ether and sodium deoxycholate. Mercuric ions and cetyltrimethyl ammonium bromide also inhibit the hydrolysis. Compared with lipase activity, this enzyme is relatively stable to heat.

The specific activity of the hydrolysis of lecithin by the lysosomal enzyme is considerably higher than those reported for mitochondrial and microsomal phospholipases. The enzyme resembles other hydrolases of the lysosome in that it has an acid pH optimum (pH 4.5). This enzymic activity is present in both the lysosomal soluble enzyme fraction and in the lysosomal membrane fraction.

The enzyme may participate in the intracellular digestion of mitochondria that is carried out by the intact lysosome in vivo. Localized inflammation and changes in vascular permeability following tissue damage could be catalyzed by this phospholipase.

KEY WORDS lysosomes · rat liver · phospholipase · lecithin · phosphatidyl ethanolamine · lysolecithin · phospholipid · mitochondria · uncoupling · inflammation

There have been several advances recently in our knowledge of the intracellular distribution and the substrate specificity of phospholipid-hydrolyzing enzymes. Phospholipase A activity (EC 3.1.1.4) has been found in rat liver in both mitochondrial and microsomal subcellular fractions (1–4). Scherphof, Waite, and Van Deenen (5) found evidence that there are two distinct types of phospholipase A activity in rat liver subcellular fractions—one in mitochondria, which resembles snake venom phospholipase A in that it cleaves phosphatides at the C$_2$ position to yield 1-monoacyl lysophosphatides, and another type in microsomes, which cleaves at the C$_1$ position to yield 2-monoacyl lysophosphatides. Both forms of phospholipase A are reported to give optimum hydrolysis under alkaline conditions (pH 8.5–9.0) (3, 4). Lysophospholipase activity (EC 3.1.1.5) has been reported to be absent from rat liver mitochondria (4) but present in microsomes (3).

In a recent communication from our laboratory we reported that the uncoupling of oxidative phosphorylation and the swelling of mitochondria induced by rat liver lysosomes were associated with the production of free fatty acids by lipolytic enzymes of the lysosome (6). In particular, a lysosomal enzyme was found which cleaved lecithin to produce free fatty acids. Since phospholipase activity was hitherto unknown in lysosomes, this enzyme was chosen for further study. The present paper reports on the properties of this phospholipid-hydrolyzing enzyme. Unlike other phospholipases of animal tissues or venoms, this enzyme cleaves both fatty acids from lecithin and phosphatidyl ethanolamine, and hydrolyzes both positional isomers of lysolecithin. Unlike other phospholipid-hydrolyzing enzymes, it is inhibited by diethyl ether and by sodium deoxycholate, and is not activated by calcium ions. In common with most lysosomal enzymes, the lysosomal phospholipase gives optimum hydrolysis at an acidic pH.

MATERIALS

Uniformly ^{14}C-labeled lecithin (specific activity 10 mc/mmole) and uniformly ^{14}C-labeled phosphatidyl ethanolamine (specific activity 5 mc/mmole) were obtained from Applied Science Laboratories Inc., State College, Pa. and were purified by chromatography on thin layers of Silica Gel G (Brinkmann Instruments Inc., Westbury, N.Y.). Two lysolecithins were prepared from lecithin-U-^{14}C. 2-Monoacyl 3-glycerophosphoryl choline-U-^{14}C was prepared by the action of pancreatic lipase according to

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the method of De Haas, Sarda, and Roger (7). 1-Monoacyl 3-glycerophosphoryl choline-U-^{14}C was prepared from lecithin-U-^{14}C by the method of Robertson and Lands (8) except that *Naja naja* venom was used. Both lysolecithins were purified by thin-layer chromatography before use.

For enzymic hydrolysis, phospholipids were dispersed in 0.25 M sucrose, 0.1 M with respect to Tris buffer [tris-(hydroxymethyl) amino methane] (pH 4.6), by sonication at 90 khertz using a Sonblaster 200 sonicator (Narda Ultrasonics Corp., Mineola, N.Y.).

Naja naja venom was obtained from Ross Allen's Reptile Institute, Inc., Silver Springs, Fla., and pig pancreatic lipase from Sigma Chemical Co., St. Louis, Mo.

METHODS

Preparation of Rat Liver Lysosomes

Male Sprague-Dawley rats (200–250 g) were starved for 24 hr and then killed by decapitation. The livers were rapidly removed and homogenized in ice-cold 0.25 M sucrose solution. The liver subcellular fractions were separated, and the lysosomes were purified by methods previously described (9, 10). To release lysosomal soluble enzymes, we froze and thawed lysosomes 10 times. Lysosomal membrane was obtained by centrifugation of the disrupted lysosomes at 105,000 *g* for 30 min; the lysosomal membrane pellet was washed once with 0.1 M NaCl and finally resuspended in 0.25 M sucrose. Protein was determined by the method of Miller (11).

Hydrolysis of ^{14}C-Labeled Phospholipids

^{14}C-Labeled phospholipids were incubated with lysosomal fractions under nitrogen at 37°C in a shaking waterbath. At the end of the incubation, unlabeled lipids, 100 μg of each, were added as carrier for the radioactive lipids. The reaction was then stopped by extraction of the lipids into chloroform by the method of Folch, Lees, and Sloane Stanley (12). The volume of chloroform was reduced to about 0.25 ml by evaporation under nitrogen and the extracted lipids were separated by chromatography on thin layers of Silica Gel G. Phospholipids were separated from fatty acids by a mobile phase of chloroform–methanol–water (65:25:4, v/v). Lipids were detected on thin-layer chromatograms by staining with iodine vapor. Appropriate areas of silicic acid were scraped off and lipids were extracted from them by the method of Abramson and Blecher (13). After removal of solvent the radioactivity of each lipid fraction was determined by liquid scintillation counting in a Packard Tri-Carb liquid scintillation spectrometer.

RESULTS

Effect of pH

The effect of pH on the hydrolysis of lecithin-U-^{14}C by lysosomal membrane is shown in Fig. 1. The production of free fatty acids is maximal at about pH 4.5. The pH range over which hydrolysis occurs is rather narrow and there is little hydrolysis above pH 6.5.

Products of the Hydrolysis of Lecithin and of Isomeric Lysolecithins

Table 1 shows the production of free fatty acids from lecithin-U-^{14}C and from two ^{14}C-labeled lysolecithins derived from lecithin-U-^{14}C by enzymic deacylation at specific positions. Although there is considerable hydrolysis of lecithin-U-^{14}C there is no production of lysolecithin-U-^{14}C. Separate experiments, not reported here, showed that the production of water-soluble radioactive products is small (less than 1% of the total radioactivity). The absence of lysolecithin-U-^{14}C from the products prompted the measurement of lysolecithinase activity under identical conditions, using both positional isomers of lysolecithin-U-^{14}C as substrates. Table 1 shows that both forms of lysolecithin-U-^{14}C are hydrolyzed by lysosomal membrane and lysosomal soluble enzymes, to a similar extent.

Effect of Suspending Medium on Hydrolysis of Lecithin

It is well known that the nature of the suspending medium greatly influences the rate of hydrolysis of phospholipids by phospholipases. In general, lecithinases require a lipophilic medium from maximum activity, and moist di-

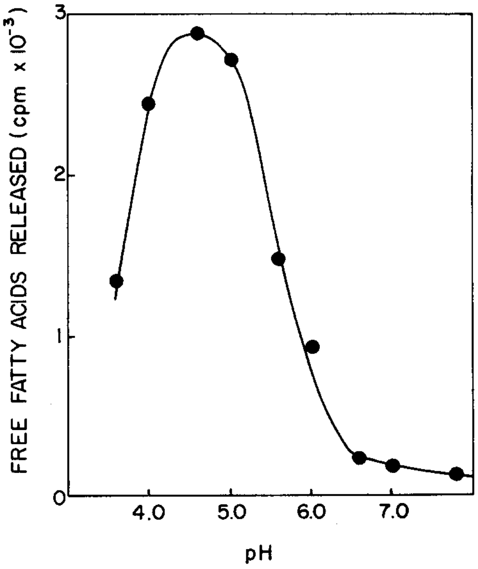

FIG. 1. Effect of pH on the hydrolysis of lecithin-U-^{14}C by lysosomes of rat liver. Each reaction mixture contained 140 μg of protein of a lysosomal membrane fraction and a suspension of lecithin-U-^{14}C (total volume of 1.0 ml) in 0.25 M sucrose in 0.1 M acetate buffer at the appropriate pH. Reaction time was 4 hr at 37°C.

TABLE 1 HYDROLYSIS OF LECITHIN AND OF TWO POSITIONAL ISOMERS OF LYSOLECITHIN BY RAT LIVER LYSOSOME FRACTIONS

Incubation Mixture	Lyso-lecithin	Lecithin	Fatty Acids
	*% of recovered radioactivity**		
Lecithin + water	5.9	92.7	2.4
	9.0	88.6	2.3
+ LM	3.5	67.9	28.6
	7.9	62.1	30.0
+ LS	4.7	30.9	64.4
	4.2	31.2	64.6
1-Monoacyl 3-GPC + water	83.1	10.0	6.9
	79.9	14.8	5.2
+ LM	69.8	13.4	16.7
	74.3	14.7	11.0
+ LS	31.0	7.3	61.6
	31.4	8.4	60.2
2-Monoacyl 3-GPC + water	92.7	3.5	3.7
	91.5	3.4	4.7
+ LM	82.0	5.2	12.8
	88.5	4.3	7.3
+ LS	47.5	2.5	50.0
	43.5	2.3	54.2

Each reaction mixture contained the appropriate ^{14}C-labeled phospholipid substrate suspended with the lysosomal protein in 0.25 M sucrose, 0.1 M with respect to Tris buffer (pH 4.6), to make a total volume of 0.2 ml. GPC, glycerophosphoryl choline; LM, lysosomal membrane (0.69 mg of protein); LS, lysosomal soluble enzymes (0.22 mg of protein). Reaction time 1 hr at 37°C.

* Percentage of radioactivity recovered from the thin-layer chromatogram in each of the three spots. The two values represent two identical, independent reactions.

TABLE 2 EFFECT OF THE SUSPENDING MEDIUM ON HYDROLYSIS OF LECITHIN-U-^{14}C

Additions to Buffer	Lyso-lecithin	Lecithin	Fatty Acids
	% of recovered radioactivity		
None	10.4	54.4	35.1
	12.4	60.4	27.2
0.25 M sucrose	12.6	57.7	29.7
	14.5	52.7	32.7
Diethyl ether	11.0	81.1	7.9
	9.6	86.2	4.2
Sodium deoxycholate	10.2	88.7	1.0
	13.3	85.2	1.5
No enzyme, no additions	4.7	94.0	1.3
	5.2	93.6	1.3

Each reaction mixture contained 22 μg of soluble lysosomal protein in a total volume of 0.2 ml of 0.1 M acetate buffer (pH 4.6). Reaction time 1 hr at 37°C. The diethyl ether medium contained 15% (v/v) 0.1 M acetate buffer and the sodium deoxycholate medium contained 5 mg of sodium deoxycholate per ml of 0.1 M acetate buffer.

ethyl ether is frequently used. Alternatively, detergents such as the bile salts are used to suspend phospholipids for maximum hydrolysis by lecithinases. Table 2 shows the effect of different suspending media on the hydrolysis of lecithin-U-^{14}C by lysosomal soluble enzymes. It can be seen that the presence of sucrose in the medium is not inhibitory. However, diethyl ether and sodium deoxycholate inhibit the hydrolysis strongly.

Effect of Possible Activators and Inhibitors

Previously studied lecithinases of animal tissues and snake venoms are known to be activated by calcium ions and to be inhibited by chelating agents such as EDTA. Fluoride, mercuric, and cyanide ions have been reported to inhibit mammalian lysolecithinase activity (14). The influence of these compounds on lysosomal phospholipase action was measured and the results are shown in Table 3. Calcium ions do not activate the hydrolysis of lecithin-U-^{14}C, which shows that the enzyme is quite distinct from previously reported lecithinases. Mercuric ions inhibit the hydrolysis and this inhibition is partially lifted by the presence of EDTA. The possibility that sulfhydryl groups are important to the hydrolysis was examined by addition of *p*-chloromercuribenzoate and of dithiothreitol to separate reaction mixtures. There is no significant inhibition by *p*-chloromercuribenzoate or activation by dithiothreitol. Dawson has reported that a lysolecithinase from the mold *Penicillium notatum* requires certain lipids or detergents for activation (15), and some of these activators have been tested in this system. As shown in Table 3, glycerophosphoryl choline has no effect on the hydrolysis of lecithin-U-^{14}C whereas the other lipids, oleic acid and tristearin, and the cationic detergent cetyltrimethyl ammonium bromide, are inhibitory. Some of the inhibitory effect of the lipids could be due to the ethanol that was used to suspend them in the reaction mixture, as this solvent is slightly inhibitory.

TABLE 3 EFFECT OF POSSIBLE ACTIVATORS AND INHIBITORS ON HYDROLYSIS OF LECITHIN-U-^{14}C

Additions	Fatty Acids Liberated*
	%
None	100
EDTA	89
Calcium chloride	95
Calcium chloride + EDTA	99
Sodium fluoride	89
Potassium cyanide	92
Mercuric acetate	13
Mercuric acetate + EDTA	65
p-Chloromercuribenzoate	109
Dithiothreitol	103
Glycerophosphoryl choline	94
Oleic acid (in ethanol)	34
Tristearin (in ethanol)	58
Cetyltrimethyl ammonium bromide	0
Ethanol	69

Each reaction mixture contained 22 μg of soluble lysosomal protein in 0.2 ml of 0.25 M sucrose in 0.1 M acetate buffer (pH 4.6). All additions were at a final concentration of 1 mM. Lipids were added in 10 μl of ethanol. Reaction time was 1 hr at 37°C.

* 100 × (% radioactivity recovered as fatty acids)/(% radioactivity recovered as fatty acids, without additions).

Heat Stability

Lipase activity is heat-labile (7), but phospholipase A activity in mammalian tissues is relatively stable to heating at 60°C (4, 16). As shown in Table 4, the phospholipid-hydrolyzing enzyme of the lysosome is resistant to heating at 60°C for 10 min. The activity is destroyed by heating at 100°C for 10 min, which provides evidence that the hydrolysis is enzyme-catalyzed. There was no loss of phospholipid-hydrolyzing activity in lysosomal fractions that were stored in the frozen state for 8 wks.

Effect of Substrate Concentration

After the addition of unlabled lecithin to lecithin-U-^{14}C, the hydrolysis of lecithin-U-^{14}C was measured at three different concentrations of lecithin. The results are shown in Table 5. The degree of hydrolysis of lecithin-U-^{14}C compared with the amounts of free fatty acids produced provides further evidence that each molecule of lecithin which is hydrolyzed releases two molecules of free fatty acids. At the two lower concentrations of substrate the degree of hydrolysis is directly proportional to substrate concentration.

Hydrolysis of Phosphatidyl Ethanolamine-U-^{14}C

Subcellular fractions of rat liver were examined for enzymic activity which would release fatty acids from phosphatidyl ethanolamine-U-^{14}C at pH 4.6. Table 6 shows that the highest specific activity is in the soluble lysosomal enzymes. This distribution is similar to that of the lecithin-hydrolyzing activity; there is a predominant concentration of phospholipase activity in the lysosomal soluble enzyme fraction, in which the specific activity is 12 times that of the lysosomal membrane, and 55 times that of the homogenate. In a separate experiment we incubated lysosomal soluble enzymes with phosphatidyl ethanolamine-U-^{14}C for 3 hr to accumulate products of the hydrolysis. Table 7 shows that free fatty acids were the major product and that lysophosphatidyl ethanolamine was not formed. Thus it appears that, as with lecithin, both fatty acids are cleaved from phosphatidyl ethanolamine. The activators of phospholipase A (calcium, diethyl ether, and sodium deoxycholate), which were ineffective or inhibitory in the hydrolysis of lecithin by lysosomal enzymes, were tested in the phosphatidyl ethanolamine hydrolysis assay. The results in Table 8 closely parallel those obtained with lecithin. None of the substances activated the hydrolysis of phosphatidyl ethanolamine and all were inhibitory to some degree.

TABLE 4 Stability of the Lecithin-Hydrolyzing Enzyme to Heat

Treatment	Distribution of Radioactivity		
	Lysolecithin	Lecithin	Fatty Acids
		%	
10 Min at 0°C	1.4*	32.9	61.0
	1.3	35.0	59.0
10 Min at 60°C	3.6	37.4	54.2
	4.0	29.1	62.1
10 Min at 100°C	0	95.8	0.5
	0	95.3	0.5

Each reaction mixture contained 44 μg of soluble lysosomal enzymes in a total volume of 0.2 ml of 0.25 M sucrose in 0.1 M acetate buffer (pH 4.6). The enzyme preparations were incubated for 10 min at the appropriate temperature before incubation in the presence of substrate for 1 hr at 37°C.

* Each value represents a separate reaction. Values were corrected for the presence of small amounts of radioactivity in the unhydrolyzed substrate which chromatographed in the same way as lysolecithin and free fatty acids.

TABLE 5 Effect of Substrate Concentration on Hydrolysis of Lecithin

Substrate Concentration	Lecithin Hydrolyzed	Fatty Acids Liberated
mg/ml	*μmoles/mg protein per hr*	
0.05	0.04	0.10
	0.04	0.12
0.50	0.36	0.85
	0.35	0.85
5.00	1.24	2.32
	1.35	2.31

Each reaction mixture contained a mixture of ^{14}C-labeled and unlabeled lecithin and 0.22 mg of lysosomal soluble proteins suspended in a total volume of 0.2 ml of 0.25 M sucrose in 0.1 M acetate buffer (pH 4.6). The two lower substrate concentrations were obtained by dilution of the highest concentration. For the calculation of specific activities it was assumed that all the esterified fatty acids of lecithin are C_{18} acids, and that there was no significant difference in the rates of hydrolysis of ^{14}C-labeled and unlabeled lecithin. Each value is for a separate 1 hr reaction.

TABLE 6 Hydrolysis of Phosphatidyl Ethanolamine-U-^{14}C by Rat Liver Subcellular Fractions

Rat Liver Fractions	Free Fatty Acids Released
	cpm/mg protein per hr
Homogenate	16.0
Nuclear fraction	7.6
Mitochondria	21.1
Microsomes	45.0
Supernatant fraction	54.3
Lysosomal membrane	72.7
Lysosomal soluble enzymes	876.0

Each reaction mixture contained a suspension of phosphatidyl ethanolamine-U-^{14}C with 20 μl of the appropriate subcellular fraction (0.05–0.50 μg of protein) in a total volume of 0.2 ml of 0.25 M sucrose containing 0.1 M acetate buffer (pH 4.6). Incubation time 1 hr at 37°C.

DISCUSSION

Earlier reports on the existence of phospholipases and lysophospholipases need reevaluation in view of the re-

TABLE 7 PRODUCTS OF THE HYDROLYSIS OF PHOSPHATIDYL ETHANOLAMINE BY RAT LIVER LYSOSOMES

Lysophosphatidyl Ethanolamine Produced	Phosphatidyl Ethanolamine Hydrolyzed	Fatty Acids Produced
	%	
0	34.3	42.0
0	35.3	48.5

Each reaction mixture contained 120 μg of soluble lysosomal enzymes in a total volume of 0.2 ml of 0.25 M sucrose in 0.1 M acetate buffer (pH 4.6). Reaction time, 3 hr at 37°C.

The values given are for two separate reactions, and represent the percentage of the total radioactivity recovered in each spot after subtraction of blanks. The blank values were obtained from incubations in which no enzymes were added.

TABLE 8 EFFECT OF THE SUSPENDING MEDIUM ON HYDROLYSIS OF PHOSPHATIDYL ETHANOLAMINE

Additions to Buffer	Fatty Acids Liberated
	% of recovered radioactivity
0.25 M Sucrose	48.5
0.25 M Sucrose, 1 mM calcium chloride	36.0
Diethyl ether	11.5
Sodium deoxycholate, 1 mg/ml	18.5
0.25 M Sucrose, no enzyme	8.6

Each reaction mixture contained 24 μg of soluble lysosomal protein in a total volume of 0.2 ml of 0.1 M acetate buffer (pH 4.6). Reaction time, 1 hr at 37°C. The diethyl ether medium contained 15% (v/v) 0.1 M acetate buffer.

cent recognition of two forms of phospholipase A (17). The best studied of these, the snake venom enzyme, is provisionally named phospholipase A_2 because it cleaves the fatty acid ester linkage at the C_2 position of 3-phosphoglycerides to yield 1-monoacyl 3-phosphoglycerides. The other type of phospholipase A, denoted phospholipase A_1, hydrolyzes 3-phosphoglycerides at the C_1 position to produce 2-monoacyl 3-phosphoglycerides. Both forms of phospholipase have been measured in rat liver, spleen, and lung (17, 18), and it was recently proposed that phospholipase A_1 is localized in rat liver microsomes whereas phospholipase A_2 is localized in rat liver mitochondria (5). Phospholipase A_1 could be responsible for the lysophospholipase activity reported in many mammalian tissues (3, 14, 16, 19, 20) because 1-monoacyl 3-phosphoglycerides were used as substrates in these determinations. Thus a mixture of phospholipases A_1 and A_2 could hydrolyze lecithin to glycerophosphoryl choline and free fatty acids. In addition, a *Penicillium notatum* enzyme has been reported by Dawson to cleave both fatty acids from lecithin, without the accumulation of lysolecithin intermediates (15).

In the present study a large number of hydrolyses of lecithin by lysosomal fractions were carried out, and only trace amounts of lysolecithin were formed, probably by contamination of the lysosomal preparation by small amounts of microsomal protein (10). Heating the lysosomal preparations at 100°C for 10 min prevented the formation of trace amounts of lysolecithin. The results of this study show that each molecule of lecithin that is hydrolyzed gives rise to two molecules of free fatty acid. There are several mechanisms by which this hydrolysis could occur. The first explanation is that four enzymes, two phospholipases A and two lysophospholipases, are responsible. Alternatively, two enzymes, phospholipase A_1 and phospholipase A_2, could jointly carry out this hydrolysis. A third explanation is that an enzyme is present in the lysosome which resembles the mold enzyme found by Dawson and which cleaves both fatty acids from lecithin.

It is unlikely that the hydrolysis of lecithin by rat liver lysosomes is due to the concerted action of several enzymes because the inhibition studies reported here did not separate the individual effects of such enzymes. For example, lysophospholipases are inhibited by diethyl ether and by sodium deoxycholate but in this system these substances caused no accumulation of lysolecithin. The lack of activation by calcium diethyl ether, or deoxycholate indicates that neither phospholipase A_1 nor phospholipase A_2 is involved. Maximum hydrolysis of lecithin by lysosomal fractions at pH 4.5 rules out the possibility that phospholipase A_1 and phospholipase A_2 are jointly responsible for the hydrolysis because the pH optima for both the mitochondrial and the microsomal phospholipase A activities are about pH 9.0. A heat-labile lipase capable of hydrolyzing phospholipids at the C_1 position has been found in human postheparin serum (21) and an acid lipase has been found in rabbit polymorphonuclear leukocyte granules (22). However, the stability of the lysosomal activity to heating at 60°C shows that heat-labile acid lipases do not participate in the hydrolysis.

Thus lysosomes of rat liver contain an enzyme which is distinct from previously reported mammalian phospholipases and lipases in that it cleaves both fatty acids from lecithin. A similar enzyme has been found in the mold *Penicillium notatum* which, under certain physicochemical conditions, cleaves both fatty acids from lecithin (15). Like the lysosomal enzyme, this mold enzyme gives maximum hydrolysis at an acid pH (pH 3.1–4.2).

The hydrolysis of phosphatidyl ethanolamine by rat liver lysosomes is very similar to the hydrolysis of lecithin. The activity is concentrated in the soluble enzymes of the lysosome. The absence of lysophosphatidyl ethanolamine from the hydrolysis products of phosphatidyl ethanolamine provides strong evidence that the enzyme that cleaves both fatty acids from lecithin acts upon phosphatidyl ethanolamine in the same manner. The hydrolysis of phosphatidyl ethanolamine by mitochondrial or microsomal phospholipases A is activated by deoxycholate or diethyl ether, and by calcium ions. The lack of activation by these substances of the hydrolysis of phosphatidyl ethanolamine by lysosomal enzymes supports the evidence for the existence of a lysosomal phospholipase which can hydrolyze both lecithin and phosphatidyl ethanolamine. Further evidence that this enzyme is lysosomal and distinct from previously reported phospholipases is seen in the optimum pH for the hydrolysis of lecithin, pH 4.5. Previous workers have searched for phospholipase activity in lysosomes but have failed to observe it (1, 3), probably because the activity of this lysosomal phospholipase is very low at neutral and alkaline pH, where the activities of phospholipases are conventionally measured.

In our preliminary investigation of the localization of the phospholipid-hydrolyzing activity within the lysosome, maximum activity appeared to be associated with the lysosomal membrane. In this study, however, the same activity was present in both the lysosomal membrane and the soluble enzymes of the lysosome and to a greater extent in the latter. The reason for this difference is not clear, but the enzyme may be activated by complex lipids of the lysosomal membrane under certain conditions, as is observed with the mold phospholipase. It is of interest that the ratio of lysophospholipase activity to phospholipase activity was greater in the lysosomal soluble enzymes than in the lysosomal membrane fraction. This difference could be due to slight contamination of the lysosomal membrane fraction with microsomes containing phospholipase A activity.

The results of Bjørnstad (3) revealed the paradox that, although the predominant activity in mitochondria was that of a phospholipase A, which resulted in the accumulation of lysophospholipids, fatty acids were liberated from both the C_1 and C_2 positions of phospholipids. There are now two possible explanations for this finding. Firstly, contamination of mitochondria with microsomes would give rise to the combined effects of phospholipase A_1 and A_2, which would liberate fatty acids from both positions of phospholipids, according to the findings of Scherphof et al. (5). An alternative explanation can be derived from the present study, because all rat liver mitochondrial preparations contain lysosomes. Lysosomes, in turn, contain partially digested mitochondria and products of the complete digestion of mitochondria, including fatty acids derived from both the C_1 and C_2 positions of mitochondrial phospholipids.

An important aspect of the present study is that the maximum specific activity of the lysosomal phospholipase is over 40 times that reported for the mitochondrial and microsomal phospholipases (4). Thus, although lysosomes comprise only about 1% of the total protein of rat liver, their total capacity to hydrolyze phospholipids could be equal to that of mitochondria and microsomes. The release of this phospholipase activity after tissue damage could result in massive changes in vascular permeability and lead to inflammation. In tissue damage lysosomal enzymes are released within the cell, and these could cause inflammation by an action analogous to that of snake and bee venom phospholipases. These enzymes disrupt membrane phospholipids, with resultant tissue swelling and alteration of the fluid balance. A protein that is released from polymorphonuclear leukocyte granules and initiates inflammation has been described by Janoff, Schaefer, Scherer, and Bean (23), and may be related to the phospholipase of this study.

There have been few studies of lipolysis by lysosomal enzymes, although the capacity of lysosomes for the digestion of intracellular structures suggests that a wide array of lipolytic hydrolases should be present in these organelles. An acid lipase (22) in rabbit leukocyte granules, a phosphatidic acid phosphatase (24), and the phospholipase described in this study provide evidence that lipids—like proteins, carbohydrates, and nucleic acids—are susceptible to catabolic reactions in the lysosome.

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