Chemistry and Physics of Lipids 15 (1975) 125–137
© North-Holland Publishing Company

STUDIES ON LYSOPHOSPHOLIPASES, V. THE ACTION OF LYSOLECITHIN-HYDROLYZING ENZYMES ON LECITHINS AND 1-ACYL LYSOLECITHINS WITH VARYING FATTY ACID CHAIN-LENGTH

J.G.N. DE JONG, R. DIJKMAN and H. VAN DEN BOSCH
Biochemistry Laboratory, University of Utrecht, Padualaan 8, Utrecht, The Netherlands

Received June 15, 1975, accepted August 4, 1975

The activity of two purified lysolecithin-hydrolyzing enzymes on homologous series of synthetic lecithins containing two identical fatty acyl chains and of 1-acyl-lysolecithins has been measured as a function of substrate concentration. In general, enzymatic activity toward lecithins decreased with increasing chain length. Maximal hydrolysis rates for the lysolecithin series were measured with 1-dodecanoyllysolecithin. In this series increased affinities for substrates with increasing acyl-chain length was noticed. In the substrate concentration *versus* enzymatic velocity curves no breaks were observed at the critical micelle concentration of the various substrates. The initial site of attack during hydrolysis of short-chain lecithins was determined using 1-octanoyl-2-pentanoyl-lecithin, 1-hexanoyl-2-hexyllecithin and 1-hexyl-2-hexanoyllecithin. Both enzymes exhibited a pronounced preference for hydrolysis of the acyl ester bond at the 1-position. Especially the enzyme from beef pancreas seems to be suitable for the enzymatic preparation of 2-acyl lysolecithins from the corresponding short-chain lecithins.

I. Introduction

In recent years we have purified the lysophospholipase activity from beef pancreas [1] and beef liver [2] to homogeneity. Both enzymes were shown to exhibit more general esterolytic properties and were able to hydrolyse short-chain lecithins [3] besides the long-chain lysolecithins which are thought to be the physiological substrates for these lysophospholipases (EC. 3.1.1.5.). Naturally occurring long-chain lecithins in the absence of detergents and surprisingly also short-chain lysolecithins were not degraded by the lysophospholipases [3]. In view of these preliminary observations we have made a more systematic study of the influence of the acyl-chain length in both lecithin and lysolecithin on the rate of hydrolysis by these lysophospholipases. Particular attention was paid to the question whether the enzymes were active on substrate monomers, micelles or both. No abrupt changes in the velocity of the enzymatic deacylation upon passing the critical micelle concentration of the various substrates were observed.

The large differences in hydrolysis rates observed for a given short-chain lecithin at one hand and the corresponding short-chain lysolecithin on the other hand pre-

dicted the enzymes to exhibit an apparent phospholipase A activity on the short-chain lecithin. This was confirmed by an analysis of the products formed during hydrolysis of dioctanoyllecithin. In addition the initial site of attack was investigated using 1-octanoyl-2-pentanoyl-glycerophosphorylcholine followed by a gaschromatographic analysis of the acylconstituent in the isolated lysophosphatidylcholine.

II. Materials and methods

A. Enzymes

The purification of the lysophospholipase from beef pancreas has been described previously [1,4]. Beef liver contained two enzymes with lysophospholipase activity, provisionally denoted as lysophospholipase I and lysophospholipase II [3]. In the experiments described in this paper purified lysophospholipase II was used.

B. Substrates

The $CdCl_2$ adduct of *sn*-glycero-3-phosphorylcholine was prepared from egg lecithin according to the method described by Chadha [5]. Short-chain lecithins were synthesized from crystalline *sn*-glycero-3-phosphorylcholine ($CdCl_2$-adduct) and the respective acylchloride as described by Baer and Buchnea [6]. The products were purified by silicic acid column chromatography according to established procedures. 1-Acyl lysolecithins were prepared from the corresponding diacyllecithins by phospholipase A_2 (*Crotalus adamanteus*) hydrolysis and purified by silicic acid chromatography. *Rac*-1-octanoyl-2-pentanoylphosphatidylcholine, *rac*-1-hexyl-2-hexanoyl-phosphatidylcholine and the isomeric *rac*-1-hexanoyl-2-hexylphosphatidylcholine were obtained by total synthesis as will be described elsewhere. Lipid phosphorus was determined by the method of Chen et al. [7] on samples ashed by the procedure described by Ames and Dubin [8].

C. Enzyme assays

The hydrolysis of lecithins and lysolecithins with acyl-chains up to dodecanoate was measured by continuous titration of the liberated fatty acids with 0.01 N NaOH at a constant pH of 7.5 using a Radiometer pH-stat TTT_2 equipment. Titration was carried out at 37°C in an incubation medium consisting of 1.0 ml of 0.1 mM potassium phosphate buffer containing 0.1 mM EDTA and 0.1 M NaCl.

In view of the low efficiency of titrating long-chain fatty acids at this pH value the deacylation of 1-[1-^{14}C] hexadecanoyllysolecithin (spec. act., 100 dpm/nmole) and 1-[9, 10-$^{3}H_2$] octadecanoyllysolecithin (spec. act., 1650 dpm/nmole) was measured by a modified Dole-extraction [9] of the released fatty acid followed by determination of radioactivity in a Packard Tricarb liquid scintillation spectrometer as described previously [1].

D. Analysis of products

The hydrolysis of short-chain lecithins by lysophospholipases was stopped by addition of trichloroacetic acid or methanol, after which the incubation mixture was evaporated to dryness. Products were dissolved in chloroform/methanol/water (65 : 35 : 8, v/v/v) and separated on silica gel G plates by development in chloroform/methanol/water (65 : 35 : 8 v/v/v). After localization of the products with the use of reference compounds, phosphorus containing products were scraped off and quantitatively determined according to Rouser [10]. In the experient examining the positional specificity of the enzymes with respect to short-chain lecithins, the degradation poducts of 1-octanoyl-2-pentanoylphosphatidyl-choline were separated on silica gel G plates. The lyso-compound was scraped off and treated with 4 ml butanol and two drops of concentrated sulfuric acid for 16 hr at 60°C. After centrifugation the butanol was collected. The silica was washed with 5 ml of heptane and the heptane combined with the butanol. This mixture was washed 3 times with 4 ml of water and then dried over sodium sulfate. An appropriate amount was injected into a Perkin Elmer FII Gas chromatograph equipped with a 12% PEGA on Gas-chrom. Q column at 95°C to separate the butyl esters of pentanoate and octanoate.

III. Results

A. Effect of acyl-chain length in lecithins

The degradation of the intermediate-chain diacyllecithins (didecanoyl- and didodecanoyllecithin) by pancreatic lysophospholipase (fig. 1) proceeded at a lower rate than that measured for the short-chain licithins (dihexanoyl- and dioctanoyllecithin). Dihexanoyllecithin forms an exception to the otherwise apparent regularity of decreasing reaction rates with increasing chain length, at least in the concentration range used. However, measurements at higher dihexanoyllecithin concentrations indicated a further linear increase in activity with substrate concentrations up to at least 20 mM (compare fig. 2). Also the liver lysophospholipase (fig. 1) exhibited lower activities when the acyl-chains were elongated. With respect to dihexanoyllecithin the same phenomenon as found for the pancreatic enzyme was observed here. Again measurements at higher concentrations of this substrate indicated a further continuous increase in enzymatic activity up to 15 mM dihexanoyllecithin (compare fig. 2). It should be mentioned that phospholipase A_2 from porcine pancreas also deacylated micellarly dispersed dihexanoyllecithin at lower rates than the corresponding structures of diheptanoyl and dioctanoyllecithin [11]. Both enzymes exhibited low activities towards didodecanoyllecithin. These results are in line with earlier observations indicating that the lysophospholipases have no hydrolytic activity *versus* naturally occurring long-chain phosphoglycerides [2,3]. The observed decrease in

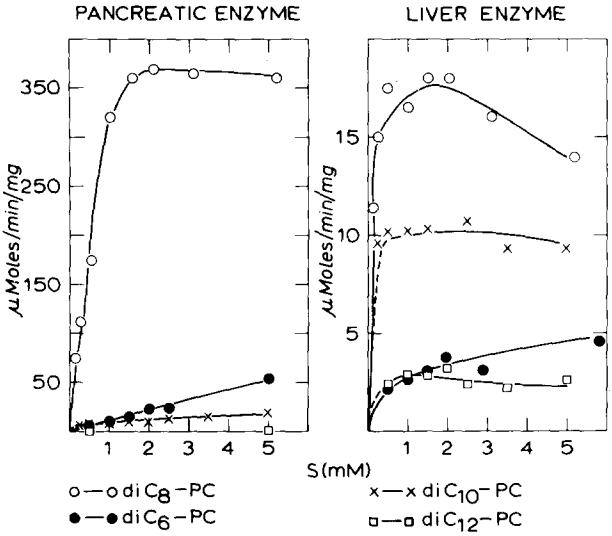

Fig. 1. Activity of lysophospholipases toward diacyllecithins with varying acyl-chain length. Substrate was sonicated in titration buffer (see Methods). To the incubation mixture (1 ml) an appropriate amount of enzyme was added. Initial velocities were measured titrimetrically at pH 7.5 and 37°C. •——• dihexanoyl-, ○——○ dioctanoyl-, X——X didecanoyl-, □——□ didodecanoyllecithin.

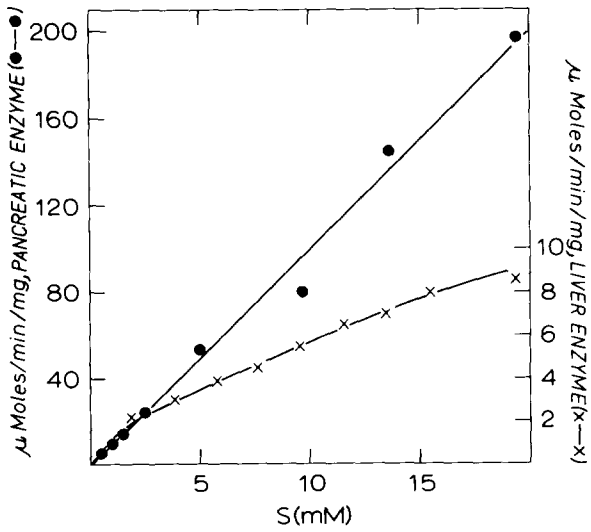

Fig. 2. Activity of lysophospholipases toward dihexanoyllecithin. For experimental details see legend of Fig. 1. •——• beef pancreatic lysophospholipase; X——X beef liver lysophospholipase.

activity of the pancreatic and liver lysophospholipase towards lecithins with increasing acyl-chain length is in agreement with trends observed by Saito and Kates [12] for the phospholipase B from *Penicillium notatum.* However, a more detailed comparison of the results is hampered by the fact that these authors did not include dihexanoyllecithin in their homologous series of lecithins ranging from dioctanoyllecithin to dioctadecanoyllecithin. Moreover, the activity of the *P. notatum* enzyme was only measured at a fixed (4 mM) concentration of the lecithin substrates.

Lipolytic enzymes often show a marked preference for organized lipid structures. This was first shown for pancreatic lipase by Sarda and Desnuelle [13] who demonstrated a sharp rise in enzymatic activity *versus* triacetin at the formation of a triglyceride–water interface. Likewise, the enzymatic activity of phospholipase A_2 from porcine pancreas sharply increased at the critical micelle concentration (CMC) of the substrates [14]. Such a pronounced increase in activity at the CMC was never observed for lysophospholipases. The CMC of dioctanoyllecithin has been reported to be 0.2 mM [14]. Although it cannot be excluded that a small plateau at this concentration of dioctanoyllecithin was missed (fig. 1), it is unlikely that enzymatic activity at the CMC changes as dramatically as described for phospholipase A_2.
Similarly, no sudden increase in the hydrolysis rate of dihexanoyllecithin at its CMC (9.5 mM) [14] was observed, neither for the pancreatic enzyme, nor for the beef liver lysophospholipase (fig. 2).

B. Effect of acyl-chain length in lysolecithins

The data in fig. 3 confirm earlier findings for pancreatic lysophospholipase [3] in-

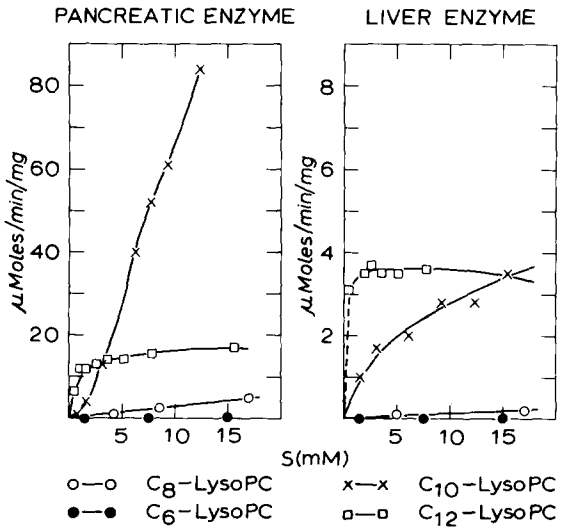

Fig. 3. Activity of lysophospholipases toward 1-acyllysolecithins with varying acyl-chain length. Conditions as for Fig. 1. ●——● 1-hexanoyllysolecithin, ○——○ 1-octanoyllysolecithin, X——X 1-decanoyllysolecithin, □——□ 1-dodecanoyllysolecithin.

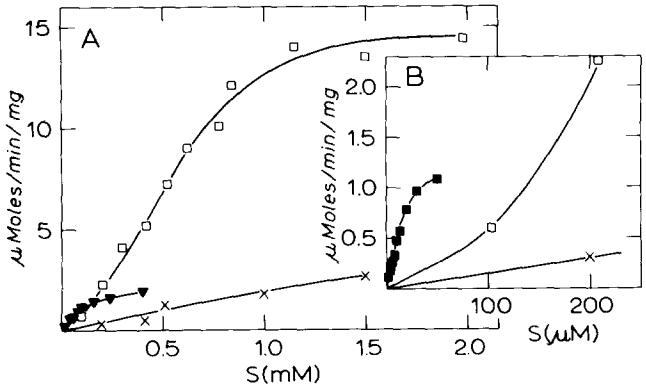

Fig. 4. Activity of lysophospholipase from beef pancreas toward lysolecithins at low substrate concentrations. Rates for hydrolysis of 1-decanoyllysolecithin and 1-dodecanoyllysolecithin were measured titrimetrically. Deacylation of hexadecanoyllysolecithin and octadecanoyllysolleci thin was measured in a radioactive assay as described under Methods. X——X 1-decanoyllysolecithin, □——□ 1-dodecanoyllysolecithin, ▲——▲ 1-hexadecanoyllysolecithin, ■——■ 1-octadecanoyllysolecithin.

dicating that the enzymic deacylation of short-chain lysolecithins proceeds at very low, barely detectable, rates. Increasing the fatty acyl-chain length led to higher activities for both enzymes. Compared with the activities on the diacyllecithins a largely reserved trend for the effect of the acyl-chain length on the hydrolysis rates of the lysolecithins is observed. Yet, a straight-forward relationship between the velocities at the highest substrate concentrations tested and the length of the acyl-chain could not be found. Perhaps the most important indication that can be obtained from fig. 3 is that of an apparent increased affinity for substrates with increased acyl-chain length.

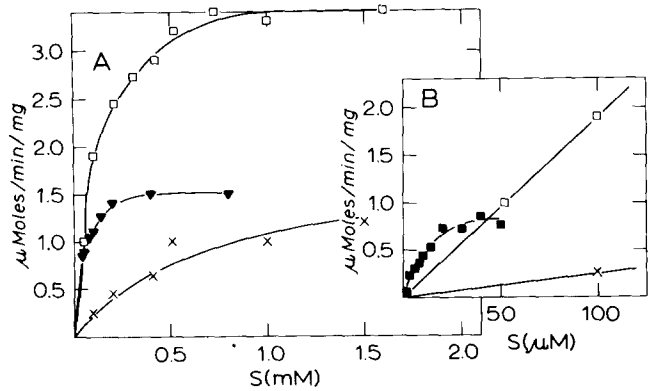

Fig. 5. Activity of beef liver lysophospholipase toward lysolecithins at low substrate concentrations. Conditions and symbols as for fig. 4.

In order to extent these studies and to enable a comparison with the naturally occurring long-chain lysolecithins hydrolysis rates were measured in the concentration range up to 1.5 mM (figs. 4A and 5A). Interpretation of the results is hampered by the fact that even over this smaller concentration range the substrates will be present in different physicochemical states, i.e. monomers and micelles.

For a fixed substrate concentration the ratio of monomers to micelles will be different for each of the substrates tested whereas on the other hand the ratio of monomers to micelles will vary for each of the substrates in the concentration range used. Therefore, in figs. 4B and 5B the rates of hydrolysis of 1-decanoyllysolecithin, 1-dodecanoyllysolecithin and 1-octadecanoyllysolecithin were plotted as a function of the substrate concentration in the monomeric region.

1-Octadecanoyllysolecithin was used in this experiment because it was available with a high enough specific radioactivity to allow accurate measurements of its hydrolysis below the CMC. These figures clearly indicate an increased affinity with increasing chain length. Moreover, in the concentration range where all substrates are present in monomeric form, i.e. below the CMC of long-chain lysolecithin (about 20 μM) [15,16,17] hydrolysis rates increase with increasing chain length. Saito and Kates [12], in measuring the enzymatic activity of the phospholipase B from *P. notatum* toward a homologous series of lysolecithins, observed decreased hydrolysis rates as the chain length increased from dodecanoate to octadecanoate. However, these authors determined enzymatic activity only at fixed lysolecithin concentrations of 2.0 and 4.0 mM, irrespective of the CMC of the substrates. As can be seen from fig. 3 no sharp increase in enzymatic activity was found for both pancreatic and liver lysophospholipase upon passing through the CMC (6.3 mM) [14] of 1-decanoyllysolecithin.

Similarly, no such changes were found at the reported CMC (0.32 mM) [14] of 1-dodecanoyllysolecithin (figs. 4, 5). Finally, in fig. 6 the activity of both enzymes

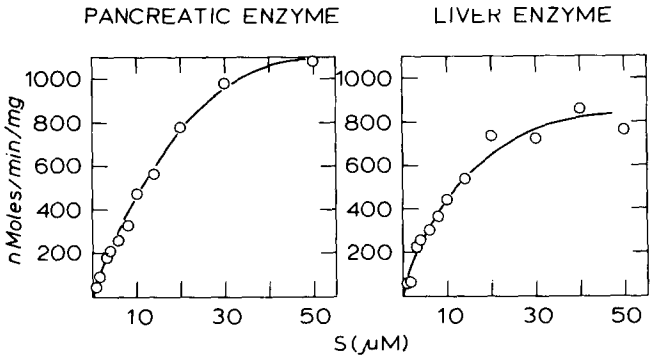

Fig. 6. Deacylation of 1-[9,10-$^3H_2$]octadecanoyllysolecithin by lysophospholipases. The indicated amounts of substrate were incubated for 10 min at 37°C with 0.12 μg pancreatic or 0.16 μg liver lysophospholipase in 0.5 ml of 20 mM potassium phosphate buffer pH 7.5.

towards monomers and micelles of 1-octadecanoyllysolecithin was measured. The CMC of this compound in the incubation medium was determined by the Wilhelmy plate technique to be 7 μM. The results clearly support the conclusion that both lysophospholipases are active on substrate monomers as well as on substrate micelles, with no indications being obtained for breaks in the substrate concentration *versus* velocity curves at the CMC of the investigated substrates.

C. Analysis of products during hydrolysis of short-chain lecithins

The large differences observed in the initial velocities for the hydrolysis of short-chain lecithins and the corresponding short-chain lysolecithins would seem to indicate that both lysophospholipases exhibit phospholipase A activity on short-chain lecithins. However, a phospholipase B activity removing both acyl constituents as observed for the pancreatic enzyme on long-chain lecithins in the presence of certain deoxycholate concentrations [4] or for the *P. notatum* enzyme [12] cannot be excluded from these titration experiments. Therefore, the degradation products after partial and complete degradation of dioctanoyllecithin by the lysophospholipases were analyzed. Hydrolysis of dioctanoyllecithin was considered to be complete when addition of more enzyme did not result in a further release of fatty acid. After partial degradation dioctanoyllecithin and octanoyllysolecithin were essentially the only phophorus-containing compounds isolated from the incubation mixture (table 1). Even after complete degradation of dioctanoyllecithin only a very small amount of glycerylphosphorylcholine was detected and phosphorus was recovered nearly quantitatively as lysolecithin (table 2). The pancreatic and liver enzyme gave identical results in this respect. The amount of fatty acid liberated during the incubation was measured titrimetrically and found to be in good agreement with the amount of octanoyllysolecithin formed (compare also table 1). This observation is in line with the large difference in activity on short-chain lecithins and the corresponding lysoderivatives (compare fig. 1 with fig. 3). Consequently both lysophospholipases exhibit an apparent specific phospholipase A activity towards short-chain lecithins. As far as the pancreatic lysophospholipase is concerned these results agree

Table 1
Partial degradation of dioctanoyllecithin by lysophospholipases from pancreas and liver.

Enzyme source	Octanoic acid (nmoles)	Glycerylphosphoryl-choline (nmoles)	Octanoyllyso-lecithin (nmoles)	Dioctanoyl-lecithin (nmoles)
pancreas	986	60	970	910
liver	997	72	995	1000

1 μg pancreatic lysophospholipase or 6 μg liver lysophospholipase II was incubated in 2 ml 0.1 mM Tris/Maleic acid buffer containing 0.1 mM EDTA and 0.1 M sodium chloride at pH 7.5 with 1860 nmoles dioctanoyllecithin. The reaction was stopped by addition of 25 μl 10% trichloroacetic acid. Products were separated and analyzed as described under Methods.

Table 2
Complete degradation of dioctanoyllecithin by lysolecithin hydrolyzing-enzymes from pancreas and liver.

Enzyme source	Glycerylphosphorylcholine		Octanoyllysolecithin		Dioctanoyllecithin	
	nmoles	%	nmoles	%	nmoles	%
pancreas	61	2.5	2360	97	13	0.5
liver	112	4.5	2360	95	15	0.5

Dioctanoyllecithin, 2600 nmoles suspended in buffer (see Table 1), was completely degraded by several additions of 2 μg pancreatic lysophospholipase or 8 μg of the liver enzyme until no fatty acid liberation could be detected titrimetrically. Products were analyzed as described under Methods.

with those of van den Bosch et al. [4], who purified a protein from beef pancreas which, in the presence of detergents, showed a phospholipase A_1 activity on long-chain lecithins and which appeared to be identical with the pancreatic lysophospholipase.

To determine the positional specificity of the phospholipase A action of the pancreatic and liver lysophospholipase on short-chain lecithins, the fatty acid composition of the lysocompound formed after complete degradation of *rac.* 1-octanoyl-2-pentanoyllecithin was investigated. The fact that the reaction can be carried to completion indicates that both lysophospholipases do not exhibit stereospecificity for the naturally-occurring derivatives of *sn*-glycero-3-phosphate, in agreement with earlier observations for the lysophospholipase from rat liver [18]. The acyl constituents in the lysocompound were converted into butylesters and analyzed by gas-liquid chromatography. The results (table 3) indicate that both enzymes preferentially liberate the octanoic acid. The small amount of octanoic acid recovered in the

Table 3
Preferential hydrolysis of octanoic acid from 1-octanoyl-2-pentanoyllecithin by lysolecithin-hydrolyzing enzymes from beef pancreas and liver.

Enzyme source	Glycerylphosphoryl-choline (%)	Lysolecithin %	Lecithin %	Octanoic acid lyso-lecithin %
pancreas	1.8	98.2	0	3.9
liver	1.4	98.6	0	6.5

1-Octanoyl-2-pentanoyllecithin (20 μmoles) was completely degraded by addition of several amounts of 10 μg lysophospholipase from beef pancreas or 40 μg beef liver lysophospholipase until no fatty acid was liberated after additon of a fresh amount of enzyme. Products were separated and analyzed as described under Methods. The fatty acid composition of the lysolecithin was determined after reesterification with butanol with the use of a gaschromatograph as described under Methods.

lyso-compound could be attributed to the fact that the original substrate contained a few percent of the isomeric lecithin with the octanoate at the 2-position. Nevertheless, these results provide no absolute proof for hydrolytic specificity at the 1-position of short-chain lecithins. Theoretically, the findings could be explained by a profound preference of both enzymes for the octanoyl group, when compared with the pentanoyl group. In order to distinguish between both possibilities, experiments were carried out with *rac* 1-hexyl-2-hexanoyllecithin and the isomeric *rac*-1-hexanoyl-2-hexyllecithin.

Assuming that the results described in table 3 would be indicative for positional specific hydrolysis one would expect 1-hexyl-2-hexanoyllecithin to be deacylated at a much lower rate than dihexanoyllecithin. The specific activity of the pancreatic enzyme on 1-hexyl-2-hexanoyllecithin was indeed found to be only 6% of that measured on dihexanoyllecithin (table 4). This is in agreement with the experiments of table 3. Likewise, the observation that 1-hexanoyl-2-hexyllecithin is hydrolysed almost 50 times faster than the isomeric 1-hexyl-2-hexanoyllecithin is in line with the previous findings (table 3) indicating a high preference for the pancreatic enzyme for hydrolyzing fatty acids at the 1-position of short-chain lecithins. This specificity for hydrolysis of the acyl ester bond at the 1-position of diacylphosphoglycerides is in agreement with the apparent phospholipase A_1 activity of this enzyme toward 1-[9,10-$^3H_2$] palmitoyl-2-[1-^{14}C] linoleoylphosphatidylethanolamine in the presence of deoxycholate [4].

For as yet unknown reasons 1-hexanoyl-2-hexyllecithin was consistently found to be hydrolysed much faster than dihexanoyllecithin, especially by the beef liver enzyme. However, from a comparison of hydrolysis rates of 1-hexyl-2-hexanoyllecithin and 1-hexanoyl-2-hexyllecithin a preference for the fatty acid at the 1-position can also be deduced for the beef liver enzyme, though this preference is somewhat less pronounced as for the pancreatic enzyme.

Table 4
Specific activities of beef pancreatic and beef liver lysophospholipase towards dihexanoyllecithin, 1-hexyl-2-hexanoyllecithin and 1-hexanoyl-2-hexyllecithin.

Substrate	Specific activity (mU/mg)	
	pancreatic lysophospholipase	liver lysophospolipase
dihexanoyllecithin	12000	2000
1-hexyl-2-hexanoyllecithin	740	2100
1-hexanoyl-2-hexyllecithin	34000	26000

Substrates, dissolved at a concentration of 2 mM in 0.1 mM potassiumphosphate buffer, pH 7.5, containing 0.1 M sodiumchloride were incubated with appropriate amounts of enzyme. Initial velocities were measured by continuous titration of the liberated fatty acid.

IV. Discussion

The effect of substrate organization on the activity of lipolytic enzymes has been investigated most extensively for pancreatic lipase [13] and pancreatic phospholipase A_2 [11,14]. Both enzymes, although able to hydrolyse slowly monomolecularly, dissolved substrates, attack organized lipid–water interfaces at much higher rates. The lysophospholipases investigated in this paper do not show this phenomenon in that only gradual increases in enzymatic activities upon passing the CMC of the various substrates are observed. In this respect the lysophospholipases behave more like carboxylesterases, the activity of which was not influenced by the appearance of a triacetin emulsion [13]. This behaviour of the lysophospholipases is in line with the esterolytic properties of these enzymes described in earlier papers [2,3]. On the other hand the large preference of pancreatic lipase for triglyceride emulsions largely disappeared if the solution contained sufficiently sodium chloride [19]. These results were explained by the formation of small micellar aggregates fulfilling the minimum requirements for proper substrate orientation long before the concentration is reached where multimolecular substrate aggregates at interfaces are formed. The micelles of triacetin that were hydrolysed efficiently by pancreatic lipase contained only a few substrate molecules. These results are quoted to indicate that the activity of lipolytic enzymes is not absolutely dependent on the presence of organized lipid-water interfaces. Substrates in monomeric, small micellar and larger multimolecular aggregates can all be hydrolysed, although according to current views an enzyme behaves more typical as a lipolytic enzyme the larger its preference for organized lipid structures. According to Brockerhoff and Jensen [20] lipolytic enzymes can be ordered in a hierarchy starting with carboxylesterases acting on water-soluble substrates and culminating in lipases and phospholipase A_2 showing a large preference for completely insoluble substrates. At intermediate levels appear hydrolases acting on lipophilic substrates in monomeric form or in loosely packed interfaces. This might explain the activity of the lysophospholipases on didecanoyllecithin and dedodecanoyllecithin (fig. 1), which will be present in liposomal-like aggregates. The higher activity found on didecanoyllecithin when compared with didodedecanoyllecithin could merely reflect a more loose packing in the liposomes of the first lipid. On the other hand, the striking differences in hydrolysis rates of dioctanoyllecithin and dihexanoyllecithin in monomeric form suggest that the length of the fatty acid chain itself influences enzymatic activity.

Leibovitz-BenGershon et al. [17] have described irregular kinetics for the lysophospholipase activity in a 20,000 X g particulate preparation a microsomal preparation and the 100 000 X g supernatant from rat brain. The substrate concentration curves for the particulate enzymes showed a maximum at concentrations greater than the CMC of the palmitoyllysolecithin used, which was dependent on the concentration of particulate pro tein. However, lysolecithin was shown to be bound for about 90% to the particles and when the reaction rates were related to the amounts of unadsorbed lysolecithin, maximal rates occurred close to the CMC of the substrate [21]. This suggested that the

membrane-bound enzymes were only active on substrate monomers and inhibited by micelles. This hypothesis could not be proven as it turned out that at the maximal rates of lysolecithin hydrolysis a constant amount of 0.25 nmole of lysolecithin per μg protein was bound, suggesting an effect of lysolecithin at the membrane level. Thus, the kinetics of membrane-bound lysophospholipase as a function of lysolecithin concentration [21] appears to be much too complicated to be compared with the experiments with the highly purified lysophospholipases described in this paper. In this respect it is interesting to note that with the partially purified lysophospholipase activity from rat brain supernatant reaction rates increased to about 80 μM palmitoyllysolecithin concentration and that no break in the curve was observed at the CMC of 17 μM [17].

The apparent phospholipase A_1 activity of the lysophospholipases on short-chain lecithins has some analogies in the recent literature about lipolytic enzymes. The pancreatic enzyme exhibits this activity also on naturally-occurring, long-chain phosphoglycerides provided these are presented in mixed micelles with deoxycholate in sufficiently high enough concentration to inhibit the lysophospholipase activity of the enzyme [4]. The phospholipase A_1 purified 5000-fold from *E. Coli* by Scandella and Kornberg [22] was twice as active on 1-acyllysophosphaditylethanolamine as on phosphatidylethanolamine and thus clearly exhibits both lysophospholipase and phospholipase A_1 activities. Raybin et al. [23] purified a phospholipase A_1 from *Bacillus megaterium* which was active only in the presence of detergents and under these conditions still exhibited lysophospholipase activity. Recently, Nishijima et al. [24] isolated an enzyme, from *Mycobacteriumphlei*, the phospholipase A_1 activity of which could be measured only in the presence of detergents, but whose capacity to degrade lysophosphatidylethanolamine required no detergents Finally, the phospholipase A_1, released by heparin treatment from rat liver plasma membranes was equally active on 1-acyllysophosphatidylethanolamine and on phosphatidylethanolamine [25].

With the exception of the latter all enzymes were highly purified justifying the conclusion that the reported activities reside in each case in a single protein. It becomes evident then that these hydrolases represent a class of nonspecific lipolytic enzymes whose activity and apparent specificity can be highly influenced by environmental conditions. In this respect it should be noted that in the absence of detergents the phospholipase A_1 activity of the lysophospholipases used in our experiments is only found on non-physiological, short-chain lecithins. Though the specific activities of both enzymes on long-chain, naturally occurring lysolecithins are lower than those measured on 1-decanoyl- and 1-dodecanoyllysolecithin, the high affinity found for these long-chain lysolecithins (figs. 4 and 5) suggest that at low substrate concentrations these naturally-occurring lysolecithins are the most favourable substrates. The activity of these lysophospholipases toward long-chain lysolecithins in natural and artificial membranes has already been established and is currently under further investigation.

Acknowledgements

The present study was carried out under the auspices of The Netherlands Foundation of Chemical Research (S.O.N.) and with financial aid from the Netherlands Foundation for the Advancement of Pure Research (Z.W.O).

References

[1] H. van den Bosch, A.J. Aarsman, J.G.N. de Jong and L.L.M. van Deenen, Biochim. Biophys. Acta 296 (1973) 94
[2] J.G.N. de Jong, H. van den Bosch, D. Rijken and L.L.M. van Deenen, Biochim. Biophys. Acta 369 (1974) 50
[3] J.G.N. de Jong, H. van den Bosch, A.J. Aarsman and L.L.M. van Deenen, Biochim. Biophys. Acta 296 (1973) 105
[4] H. van den Bosch, A.J. Aarsman and L.L.M. van Deenen, Biochim. Biophys. Acta 348 (1974) 197
[5] J.S. Chadha, Chem. Phys. Lipids 4 (1970) 104
[6] E. Baer and D. Buchnea, Can. J. Biochem. Physiol. 37 (1959) 953
[7] P.S. Chen, T.Y. Toribara and H. Warner, Anal. Chem. 28 (1956) 1756
[8] B.N. Ames and D.T. Dubin, J. Biol. Chem. 235 (1960) 769
[9] S.A. Ibrahim, Biochim. Biophys. Acta 137 (1967) 413
[10] G. Rouser, J. Chrom. Sci. 11 (1973) 96
[11] G.H. de Haas, P.P.M. Bonsen, W.A. Pieterson and L.L.M. van Deenen, Biochim. Biophys. Acta 239 (1971) 252
[12] K. Saito and M. Kates, Biochim. Biophys. Acta 369 (1974) 245
[13] L. Sarda and P. Desnuelle, Biochim. Biophys. Acta 30 (1958) 513
[14] W.A. Pieterson, Thesis, 1973, Utrecht
[15] R.E. Barden and W.W. Cleland, J. Biol. Chem. 244 (1969) 3677
[16] M. Robinson and L. Saunders, J. Pharm. Pharmacol. 11 (1959) 155 T
[17] Z. Leibovitz-BenGershon, I. Kobiler and S. Gatt, J. Biol. Chem. 247 (1972) 6840
[18] H. van den Bosch, A.J. Aarsman, A.J. Slotboom and L.L.M. van Deenen, Biochim. Biophys. Acta 164 (1968) 215
[19] B. Entressangles and P. Desnuelle, Biochim. Biophys. Acta 159 (1968) 285
[20] H. Brockerhoff and R.G. Jensen, in: Lipolytic enzymes, Academic Press, New York (1974) 289
[21] Z. Leibovitz-BenGershon and S. Gatt, J. Biol. Chem. 249 (1974) 1525
[22] C.J. Scandella and A. Kornberg, Biochemistry 10 (1971) 4447.
[23] D.M. Raybin, L.L. Bertsch and A. Kornberg, Biochemistry 11 (1972) 1754
[24] M. Nishijima, Y. Akamatsu and S. Nojima, J. Biol. Chem. 249 (1974) 5658
[25] M. Waite and P. Sisson, J. Biol. Chem. 248 (1973) 7985