

PRELIMINARY NOTES

BBA 51045

Formation of lysophosphatidyl ethanolamines in cell fractions of rat liver

Rat-liver homogenates have been shown to be capable of producing two isomeric lyso derivatives from added radioactive phospholipids¹. The distribution of the enzymes catalyzing these hydrolyses among the subcellular fractions was the subject of this investigation. Since the previously observed phospholipase activity of mitochondria and microsomes has been found to hydrolyze PE more rapidly than PC^{2,3} the present investigations were performed using PE instead of PC.

The substrate was prepared by mixing 1-[9,10-³H]palmityl-PE (synthesized by incubating [³H]palmitic acid, 2-acyl lyso-PE, ATP and CoA with rat-liver homogenate) with 2-[1-¹⁴C] linoleyl-PE (synthesized by incubating [¹⁴C]linoleic acid, 1-acyl lyso-PE, ATP and CoA with rat-liver homogenate). Hydrolysis of the two radioactive products by phospholipase A (EC 3.1.1.4) from the venom of *Crotalus adamanteus* indicated that 98% of the [¹⁴C]linoleic acid was in the 2-position and that 88% of the [³H]palmitate was in the 1-position. On thin-layer chromatography the labeled PE was shown to be 99% pure.

The various cell fractions were prepared as follows: A 10% rat-liver homogenate in 0.25 M sucrose was centrifuged at 1000 × g for 5 min, the precipitate being designated the nuclear fraction.

The supernatant was then centrifuged at 4500 × g for 10 min; the precipitate was resuspended and recentrifuged at 12500 × g for 10 min, the precipitate being the mitochondria. The supernatant from the 4500 × g centrifugation was then centrifuged at 12500 × g, the precipitate being designated the composite fraction, while the supernatant was recentrifuged at 100000 × g for 1 h, the precipitate being the microsomes and the supernatant the soluble fraction.

Table I indicates the extent of formation of lyso-PE, free fatty acid and GPE by the cell fractions. The homogenate was found to accumulate considerable amounts of both 1- and 2-acyl lyso-PE, basically in agreement with the findings of VAN DEN BOSCH¹. The nuclear fraction also catalyzed the hydrolysis of both fatty acids from the PE as shown by the accumulation of both lyso isomers. This fraction also contained an active lysophospholipase activity as indicated by the non-stoichiometric relationship between the amounts of free fatty acid and lyso-PE. It is not known to what this activity is due, since this fraction contains, in addition to nuclei, cell membranes and unbroken cells. The mitochondria, in comparison with the homogenate, were found to be rather specific in catalyzing hydrolysis at the 2-position as shown by the high ratio in the lyso-PE fraction and conversely, the low ratio in the fatty acid fraction. In contrast, the microsomal fraction accumulated only the [¹⁴C]lyso-PE

Abbreviations: PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; GPE, glyceryl-phosphoryl ethanolamine.

TABLE I

HYDROLYSIS OF 1-[9,10-³H]PALMITYL-2-[1-¹⁴C]LINOLEYL-PE BY SUBCELLULAR FRACTIONS FROM RAT-LIVER HOMOGENATES

Incubation mixtures contained the PE derivative (440000 disintegrations/min ³H; 47000 disintegrations/min ¹⁴C) sonicated in isotonic Tris-KCl buffer (pH 7.4) and 13 mg protein of the various cell fractions resuspended in isotonic Tris-KCl buffer (pH 7.4) (except for homogenate and soluble fraction which were in 0.25 M sucrose). Incubations were carried out at 37° for 1 h and were stopped by the addition of methanol and chloroform. The lipids were extracted by the method of BLIGH AND DYER⁷ and were separated by thin-layer chromatography (using first the chloroform-light petroleum-acetic acid system (65:35:2, by vol.) followed by the chloroform-methanol-water system (65:35:4, by vol.)). The lipids were located by staining with iodine vapour, the silicic acid scraped from the plates and the lipids extracted from the silicic acid with methanol. The ³H and ¹⁴C activities were measured in a Packard Tricarb scintillation spectrometer. All values were corrected for a control sample with boiled enzyme.

Fraction	Lyso-PE		Free fatty acid		GPE*
	Percent formed	% ³ H isomer % ¹⁴ C isomer	Percent formed	% [³ H]palmitic acid % [¹⁴ C]linoleic acid	
Homogenate	11.5	0.018	24.5	3.69	13.0
Nuclear	17.3	0.085	45.5	2.46	28.2
Mitochondrial	13.8	16.2	24.5	0.305	10.7
Composite	9.0	0.193	16.6	1.20	7.6
Microsomal	10.6	0**	37.2	3.45	26.6
Soluble	2.4	0**	28.4	2.32	26.0

* Calculated as the difference between the amount of free fatty acid and the amount of lyso-PE formed. Control experiments showed that under the conditions used there were minimal competing reactions *e.g.*, reacylating or oxidation of free fatty acid.

** No accumulation of [³H]lyso-PE was observed in these experiments.

indicating that it contains an enzyme catalyzing the hydrolysis at the 1-position. In addition the microsomes have a rather high lysophospholipase activity as can be seen from the amount of GPE formed. The composite fraction which probably contains both mitochondria and microsomes in addition to lysosomes, catalyzed the formation of a mixture of the lyso-PE isomers as would be expected. The supernatant was found to catalyze the release of both fatty acids; but due to the lack of accumulation of lyso-PE caused by its high lysophospholipase activity⁴, it is not possible to ascertain the initial site of hydrolysis.

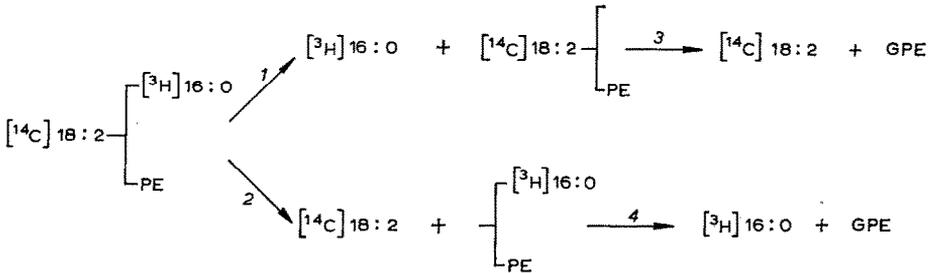
Since the relatively pure fractions of mitochondria and microsomes were found to accumulate high amounts of lyso-PE and appeared to contain enzymes with different specificities, additional studies were done using these two fractions. Protein

TABLE II

SPECIFICITY OF HYDROLYSIS OF 1-[9,10-³H]PALMITYL-2-[1-¹⁴C]LINOLEYL-PE BY MITOCHONDRIA AND MICROSOSES
Experimental conditions same as Table I.

Fraction	Percent of each isotope recovered after hydrolysis of PE				Percent of lyso-PE isomer	
	Lyso-PE		Fatty acid released		[³ H]lyso-PE	[¹⁴ C]lyso-PE
	[³ H]lyso-PE	[¹⁴ C]lyso-PE	[³ H]palmitic acid	[¹⁴ C]linoleic acid		
Mitochondria	20.9	2.3	4.4	29.5	90.0	10.0
Sonicated mitochondria	30.6	1.2	6.1	36.3	96.1	3.9
Microsomes	0.3	14.4	25.3	8.7	2.1	97.9
Sonicated microsomes	0.9	5.9	9.4	4.2	13.3	86.7

concentrations and times giving optimal activity were determined and used throughout these experiments. It was found that sonication of the mitochondria stimulated the activity, particularly that giving rise to the formation of tritiated lyso-PE (Table II). The microsomal fraction, on the other hand, had decreased activity upon sonication. Possibly this decrease in activity is due to inactivation of the enzyme or to the increase in endogenous lipid available for hydrolysis.



Scheme 1. Possible conversions of 1-[9,10-³H]palmityl-2-[1-¹⁴C]linoleyl-PE.

Furthermore, it can be seen that the lyso-PE recovered after the action of the mitochondria is more than 90% the ³H-labeled lyso isomer. It is possible that this [³H]lyso-PE might contain a small amount of the 2-isomer since 12% of the ³H-labeled fatty acid was located at the 2-position. These data clearly indicate that the main site of hydrolysis catalyzed by the mitochondria is the same as the site of action of phospholipase A from snake venom⁵ or the pancreas phospholipase A (ref. 6). The ³H-labeled fatty acid formed (about 5%) could be due to the presence of an enzyme catalyzing the hydrolysis at the 1-position of the PE (Reaction 1) followed by the action of a lysophospholipase on the [¹⁴C]lyso-PE accounting for the low amount of [¹⁴C]lyso-PE (Reaction 3), or due to the further hydrolysis of the [³H]lyso-PE (Reaction 4). Further, a certain amount of ³H-labeled free fatty acid would be expected to arise from the 2-position of the PE by the action of a phospholipase A. Experiments similar to these using deoxycholate to inhibit lysophospholipase activity indicate the latter case to be more probable; hence Reaction 2 solely accounting for hydrolysis of the PE.

The action of the microsomes, however, gives rise to lyso-PE which is about 90% the ¹⁴C-labeled isomer. This represents the minimal amount of the 2-acyl lyso-PE present since 12% of the palmitate in the PE is in the 2-position. Due to the relatively high activity of a lysophospholipase in the microsomes it is more difficult to ascertain the specificity of the hydrolysis of the PE causing the formation of high amounts of fatty acids. It is possible that the PE is hydrolyzed to an almost equal extent at the 1- and 2-positions (Reactions 1 and 2) followed by the hydrolysis of the [³H]lyso-PE by a lysophospholipase (Reaction 4). On the other hand, it is possible that the microsomal enzyme is highly specific in hydrolyzing at the 1-position (Reaction 1) since little [³H]lyso-PE is formed (this amount, 1.4%, could be in the 2-position) followed by the action of a lysophospholipase (Reaction 3).

From the present data it can be concluded that the phospholipase activities in the rat liver catalyzing the hydrolysis at the 1- or 2-positions are not randomly

distributed among the cell fractions. Evidence presented here indicates that the mitochondria contain mainly the phospholipase A whereas the microsomes contain mainly an enzyme catalyzing the hydrolysis of PE at the 1-position. The extent to which these different lyso derivatives play a role as acyl acceptors for saturated and unsaturated fatty acids in cell fractions is currently under investigation.

The work of M. W. was made possible through an Advanced Research Fellowship of the American Heart Association. The present investigations have been carried out under the auspices of the Netherlands Foundation for Chemical Research (S.O.N.) and with financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.). The authors wish to thank Mr. A. J. AARSMAN for technical assistance.

*Department of Biochemistry,
The State University, Utrecht (The Netherlands)*

G. L. SCHERPHOF
MOSELEY WAITE
L. L. M. VAN DEENEN

- 1 H. VAN DEN BOSCH AND L. L. M. VAN DEENEN, *Biochim. Biophys. Acta*, 106 (1965) 326.
- 2 G. L. SCHERPHOF AND L. L. M. VAN DEENEN, *Biochim. Biophys. Acta*, 98 (1965) 204.
- 3 P. BJÖRNSTAD, *Biochim. Biophys. Acta*, 116 (1966) 500.
- 4 G. V. MARINETTI, J. ERBLAND, R. F. WITTER, J. PETIX AND E. STOTZ, *Biochim. Biophys. Acta*, 30 (1958) 223.
- 5 L. L. M. VAN DEENEN AND G. H. DE HAAS, *Biochim. Biophys. Acta*, 70 (1963) 538.
- 6 H. VAN DEN BOSCH, N. M. POSTEMA, G. H. DE HAAS AND L. L. M. VAN DEENEN, *Biochim. Biophys. Acta*, 98 (1965) 657.
- 7 E. G. BLIGH AND W. J. DYER, *Can. J. Biochem. Physiol.*, 37 (1959) 911.

Received May 12th, 1966

Biochim. Biophys. Acta, 125 (1966) 406-409

BBA 51046

Phosphoinositide inositolphosphohydrolase in guinea-pig intestinal mucosa

The phosphatidyl inositol of the small intestinal mucosa is readily labelled *in vivo* after the administration of ^{32}P orthophosphate to rats¹. Since rapid synthesis and breakdown of the lipid takes place, it seemed likely that the mucosa would contain a phospholipase attacking inositol lipids. The presence of such an enzyme, of the phospholipase C type, is now reported.

Phosphatidyl inositol for use as substrate was prepared from crude corn phosphatidyl inositol² which was purified on alumina³ and silicic acid⁴ columns. A solution of the lipid in chloroform-methanol (2:1, by vol.) was shaken with 0.2 volume of 0.1 M sodium EDTA to remove Ca^{2+} . The resulting chloroform layer was then shaken twice with 0.2 volume of 0.9% NaCl. This washing produced calcium-free lipid as determined by a modification of the method of WILLIAMS AND WILSON⁵. The chloro-

Biochim. Biophys. Acta, 125 (1966) 409-412