BBA 75970

DISTRIBUTION OF MEMBRANE-CONFINED PHOSPHOLIPASES A IN THE RAT HEPATOCYTE

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(Received January 17th, 1972)

SUMMARY

1. A study has been made of the occurrence of phospholipases A in rat liver mitochondria, lysosomes, microsomes and plasma membrane. Some related observations on the reacylation of mitochondrial phospholipids are also presented.

2. Soluble phospholipases (pH optimum 4.5) occur only in lysosomes. Membrane-bound phospholipases in mitochondria, microsomes, plasma membrane and lysosomes have an alkaline pH optimum (pH 8.5–9.0).

3. The soluble lysosomal phospholipase activity is inhibited by KCl + $CaCl_2$. In contrast, the membrane-bound phospholipases are all stimulated by KCl + $CaCl_2$, there being an absolute requirement for Ca^{2+} in the case of mitochondrial and plasma-membrane activities.

4. The acid phospholipases in lysosomes show A_1 and A_2 specificity. The alkaline phospholipases in microsomes and plasma membrane have a predominant A_1 specificity while that in mitochondria is an A_2 phospholipase.

5. The membrane-bound enzymes show characteristic differences in affinity for exogenous substrate. The affinity for exogenous phosphatidylethanolamine is higher in plasma membrane (K_m = 40 μM) and outer mitochondrial membrane (90 μM) than in inner mitochondrial membrane and rough endoplasmic reticulum (K_m = 400 μM). In mitochondria, endogenous phosphatidylethanolamine is hydrolyzed preferentially to added [^{32}P]phosphatidylethanolamine.

6. The distribution of phospholipase A activity was studied in inner and outer mitochondrial membranes isolated by two different procedures. Although the distribution of phospholipase A_2 follows that of monoamine oxidase a significant activity was found in isolated inner membrane freed of matrix proteins. The mitochondrial phospholipase A_2 cannot be exclusively attributed to the outer membrane.

7. The labelling, by [^{14}C]oleic acid, of mitochondrial membrane phosphatidylethanolamine and lecithin can be accounted for by microsomal contamination of the mitochondria. In contrast, microsomes were less active than mitochondrial outer membrane in the synthesis of phosphatidic acid from [^{32}P]ATP.

8. Acylation of glycerophosphate produced phosphatidic acid with microsomes and lysophosphatidic acid with outer mitochondrial membrane. Accumulation of lysophosphatidic acid is attributed to a lack of acylating enzyme in the outer membrane rather than to the result of mitochondrial phospholipase action.

9. These results, in agreement with some others, point to a relatively passive role of mitochondria *vis-à-vis* structure like endoplasmic reticulum in the renewal of

cellular phospholipids: both as concerns an inability to attack exogenous substrate as well as a failure to re-acylate major structural components such as phosphatidylcholine and phosphatidylethanolamine. An exception to this rule may, however, be found in pathways involving minor "non-structural" components such as lysophosphatidic acid.

INTRODUCTION

All membrane systems that can be isolated from rat liver have been shown to contain phospholipase A activities. On the other hand, many points concerning the precise distribution of the two phospholipases A_1 and A_2 still require clarification. Most importantly, the significance of these enzymes in the physiology of the cell remains to be elucidated.

Our interest in the problem began with the observation that "aging" of mitochondria in isotonic sucrose, which results in uncoupling of oxidative phosphorylation[1,2] was accompanied by accumulation of the lyso forms of some phospholipids such as lecithin and phosphatidylethanolamine but not of others like phosphatidylinositol[1]. Some lipids, *e.g.* CoA-esters and phosphatidylinositol, were able to prolong the "life" of the mitochondria in isotonic sucrose, pointing to a possibly decisive role of phospholipase A in energy-coupling reactions in mitochondria[1,3]. In order to evaluate such a possibility, it is essential to be able to define the activity(ies) endogenous to mitochondria as compared with other membrane systems of the cell.

Our previous reports[4–7] described some differences between mitochondrial and lysosomal phospholipase A activities. The present results extend the comparison of mitochondrial phospholipase A to other subcellular fractions in particular the microsomes and the plasma membrane which are also endowed with phospholipase A activity. An evaluation of these results, based on a critical appraisal of the purity of the organelle preparations used, is made with respect to those of other laboratories obtained by different approaches describing the phospholipase A activities of mitochondria[8–12] and mitochondrial membranes[13], microsomes[11,14], lysosomes[12,15–19] and plasma membrane[20–23]. We include as well some observations on the reacylation of mitochondrial lysophospholipids.

MATERIALS

Cytochrome *c* (Type VI) and AMP were obtained from Sigma (St. Louis, Mo., U.S.A.), NADPH from Boerhinger (Mannheim, Germany), benzylamine sulfate and *p*-nitrophenylphosphate from Prolabo (France), Silica gel G and precoated plates for thin-layer chromatography from Merck (Darmstadt, Germany), Triton *X*-100 and Triton WR-1339 from Rohm and Haas (Philadelphia, Pa., U.S.A.), Digitonin (A grade) from Calbiochem (Los Angeles, Calif. U.S.A.), CoA from Pabst Laboratories (Milwaukee, Wisc., U.S.A.), [^{14}C]oleic acid and ^{32}P-labelled phosphate from C.E.A. (Saclay, France). Authentic samples of fatty acid methyl esters were purchased from Applied Science (State College, Pa., U.S.A.).

METHODS

Tissue fractionation

Mitochondria. Mitochondria were isolated from adult albino rat liver by the classical differential centrifugation method in 0.27 M sucrose buffered by 3 mM Tris–HCl, pH 7.4, from a 10% homogenate (w/v). After removal of nuclei and cell debris at 600 × *g* for 10 min in a SS-1 Servall centrifuge, mitochondria were sedimented at 5000 × *g* for 10 min, then washed three times in half-starting volume of 0.27 M sucrose–Tris mixture at 6000 × *g* for 10 min.

To get rid of most of the contaminant membranes two methods of purification were used. They have already been described[25]. The first one used Triton WR-1339 to make lysosomes lighter than mitochondria. The second method used digitonin (25 μg digitonin per mg protein, 10 min at 0 °C) which binds to and breaks cholesterol-rich membranes such as lysosome and plasma membranes. After fragmentation, these membranes can then be eliminated by differential centrifugation.

Mitochondrial membranes. Mitochondrial membranes were obtained according to Schnaitman and Greenawalt[26] or according to Parsons *et al.*[27] as already described[25]. In the second case[27] the crude outer membranes contained in the swelling fluid were collected at 20000 rev./min for 20 min in a Spinco rotor 30. This "high-speed pellet" was not further purified since, in our hands[5,25,28] it seemed better to purify first the mitochondrial preparation before separating the mitochondrial membranes. The inner membrane was obtained from the inner membrane *plus* matrix particles by two successive swellings in 20 mM phosphate buffer, pH 7.4, (10 min at 0 °C with stirring). Inner membrane vesicles were purified on the same three-layer sucrose gradient used to purify inner membrane *plus* matrix particles but were collected between the 51.3 and 37.7% (w/v) sucrose layers. The membranes were then washed with 20 mM phosphate buffer, pH 7.4, and collected by sedimentation at 42000 rev./min for 10 min.

Microsomes. Before sedimenting the microsomes, an intermediate fraction (L-fraction) was spun down by centrifugation of the post-mitochondrial supernatant fluid at 21000 rev./min. for 15 min in a SS-1 Servall centrifuge. Microsomes were then sedimented at 105000 × *g* for 40 min in a Spinco rotor 30. A soluble microsomal fraction is obtained by washing the microsomal suspension (at about 40 mg/ml) with 4 vol. of 2 mM Tris–HCl buffer, pH 7.8 and, after 10 min. at 0 °C, centrifugation at 30000 rev./min. in a Spinco rotor 30. Rough endoplasmic reticulum was obtained according to the procedure of Molnar[29], slightly modified[25].

Lysosomes. Lysosomes were isolated as tritosomes (Triton WR-1339-filled lysosomes). The soluble and membrane fractions from tritosomes were obtained by dialysis against large volumes of 1 mM $NaHCO_3$, 1 mM EDTA, pH 7.5, for 12 h[19]. The membranes were gathered by centrifugation in a Spinco rotor 40 at 35000 rev./min for 20 min. Soluble lysosomal enzymes were also obtained in S-fractions prepared from mitochondria preparations sedimented at higher speed than usual (10000 × *g* min) in order to collect more lysosomes.

Plasma membranes. Plasma membranes were isolated according to Neville[30].

Enzyme assays

To assay membrane-bound enzymes, the membranes were lysed first with

Triton X-100 (final concentration between 0.01 and 0.05% depending on the enzyme tested).

Acid phosphatase (EC 3.1.3.2.) as lysosomal marker, was assayed with *p*-nitrophenylphosphate as substrate[31], monoamine oxidase (EC 1.4.3.4), outer mitochondrial membrane marker, according to Tabor *et al.*[32], cytochrome *c* oxidase (EC 1.9.3.1), inner mitochondrial membrane marker, according to Appelmans *et al.*[33], NADPH–cytochrome *c* reductase (EC 1.6.2.3), microsomal marker, according to Omura *et al.*[34], 5'-mononucleotidase (EC 3.1.3.5), plasma membrane marker, according to Emmelot *et al.*[35]. Inorganic phosphate was measured by the method of Fiske and SubbaRow[36]. Protein was determined either by the biuret method[37], a correction for turbidity being made according to Kröger and Klingenberg[38], or according to Lowry *et al.*[39].

Preparation of [^{32}P]phosphatidylethanolamine

Rat liver [^{32}P]phosphatidylethanolamine was isolated from rats injected intraperitoneally with 1 mCi of [^{32}P]phosphate. 8–10 h after injection, the rats were killed with ether and the liver phosphatidylethanolamine isolated by conventional silicic acid chromatography. The purity of the [^{32}P]phosphatidylethanolamine peak (eluted by the chloroform–methanol mixtures (7:1 and 7:3, v/v)) was checked by thin-layer chromatography and the spots colored by spraying ninhydrin in aqueous butanol, molybdic acid in H_2SO_4 solution and finally charring-of the plate. Only the fractions giving a single ninhydrin-positive spot were pooled and after concentration of the solution another thin-layer chromatogram was carried out together with a radioactivity and phosphorus determination on the concentrated solution and on the spot isolated by thin-layer chromatography; in addition an autoradiogram of the plate was taken to detect the presence of any radioactive impurities. On the basis of radioactivity and phosphorus determination, [^{32}P]phosphatidylethanolamine was found 96% pure and [^{32}P]phosphatidylcholine 99% pure. Starting from 2 rats, 60 mg of [^{32}P]phosphatidylethanolamine (spec. act. 215000 dpm./mole) and 115 mg of [^{32}P]-phosphatidylcholine (spec. act. 171000 dpm./mole) could be obtained.

Phospholipase A

Phospholipase A activity was measured as the amounts of fatty acids and lysophosphatides formed either from endogenous membrane phospholipids or with exogenous rat liver [^{32}P]phosphatidylethanolamine which had been emulsified in 5–10 ml of ice-cold water containing 5% of ethylether (final ethyl ether concentration in the test 1%) by brief sonication (30 s twice) with a Branson sonifier set at 8 (in these conditions the amount of lysophosphatidylethanolamine formed by sonic irradiation was less than 5%). The incubation mixture was made of 20 mM buffer (acetate for pH lower than 5.5, Tris–maleate from pH 5.6 to 8.9, glycine–NaOH above pH 9), 100 to 500 μM [^{32}P]phosphatidylethanolamine, 50–300 μg of protein and, above pH 6, 2 mM $CaCl_2$ and 0.1–0.15 M KCl, in a total volume of 1 ml. For fatty acid analysis the assay was run in 2 ml of incubation medium. The fatty acids were extracted according to Dole[40] and measured after methylation by diazomethane by gas–liquid chromatography on a 2 m × 0.125 inch column packed with 20% diethyleneglycol succinate on chromosorb WAW (100 mesh) and operated at 180 °C, carrier N_2, using a Hewlett-Packard 5750 chromatograph equipped with ionisation flame detectors and with an

integrator Hewlett-Packard 3370. The internal standard used was heptadecanoic acid (17:0). The fatty acid release from exogenous phosphatidylethanolamine is given after deduction of the fatty acids released from endogenous lipids. The phospholipids were extracted by a mixture of chloroform–methanol (2:1, v/v)[41] after acidification with 1 M HCl and separated by thin-layer chromatography in the following system[42]: chloroform–light petroleum (b.p. 60 °C)–acetic acid (65:35:2, v/v/v), then, after drying the plate, chloroform–methanol–water (65:35:4, v/v/v). The radioactive spots are localized by autoradiography or ninhydrin spray and then scrapped off the plates and transferred to scintillation counting vials. The scintillation mixture was: 6 g PPO, 0.3 g POPOP, 100 g naphtalein, 50 ml ethanol, 1 l dioxane. The samples were counted in a Nuclear-Chicago Unilux II scintillation counter. Alternatively when silica gel-precoated aluminium sheets were used the spots were cut out and counted in a gas flow counter (RA 15, Intertechnique, France).

Synthesis of phosphatidic acid

The radioactivity incorporated into endogenous phopholipids after incubation with [^{14}C]oleic acid or [^{32}P]ATP (prepared according to Glynn and Chappell[43]) was estimated after extraction of the lipids, isolation of each individual phospholipid by thin-layer chromatography, localization of the spots by autoradiography, then scrapping off the spots into scintillation counting vials. The radioactive phosphatidic acid spots were separated by thin-layer chromatography in the following solvent system: chloroform–methanol–acetic acid–water (65:15:8:0.3, by vol.)[44] their migration behaviour was compared to that of authentic phosphatidic acid (gift of Dr M. Faure) and phosphatidylglycerol (gift of Dr E. Barbu). The incubation conditions are specified in each table or figure.

RESULTS

Resolution and identification of membrane fractions with reference to marker enzymes

Fig. 1 summarizes the two methods used to isolate, from rat liver homogenates,

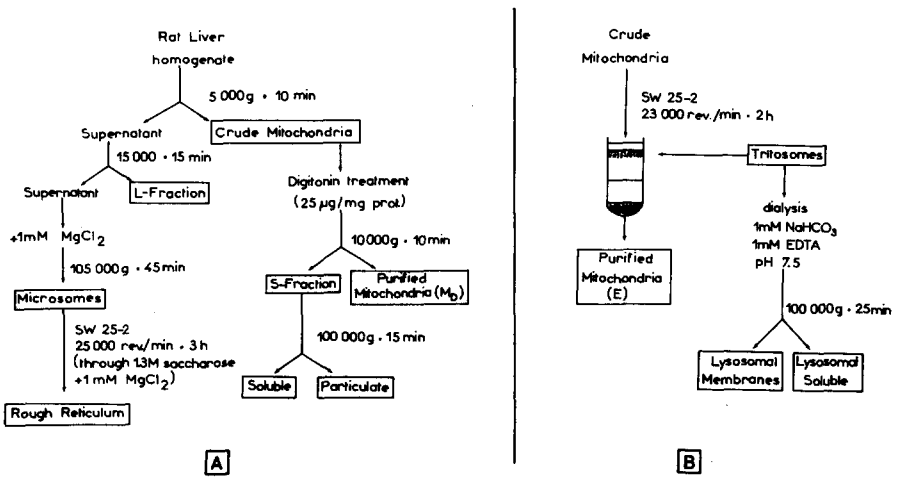

Fig. 1. Flow sheet for purification of mitochondria and isolation of subcellular membranes from normal rat liver (A) and from liver of rats previously injected with Triton WR-1339 (B).

different types of organelles known to possess a phospholipase activity. It is to be noted that after a "slight digitonin treatment" mitochondria are not so easily fractionated into inner and outer membrane (more inner membrane remains in the outer membrane fraction for instance). To recover from mitochondria enough outer membrane protein and to minimize the contamination by inner membrane, the Parsons method was applied to preparations of mitochondria which had been carefully washed three times with isotonic sucrose. Rough endoplasmic reticulum isolated in the presence of 1 mM $MgCl_2$ was found to be less contaminated by other subcellular membranes[25] than the smooth reticulum and was therefore chosen to study the phospholipase A of microsomal origin. Lysosome-enriched fractions were obtained either as L-fraction or S-fraction or tritosomes, and were further subfractionated into their soluble and particulate components as illustrated in Fig. 1.

Table I gives the specific activities of marker enzymes in the above mentioned particles and in plasma membrane used in this study. The activity of galactosyltransferase, a marker enzyme for Golgi membranes[45] is given for plasma membrane.

TABLE I

IDENTIFICATION OF MEMBRANE FRACTIONS WITH REFERENCE TO MARKER ENZYMES

Activities in nmoles·min^{-1}·mg^{-1}. N.D.: not detected.

Marker enzyme	*Mitochondria*			*Tritosome*		
	M$_{\eta}$	*Inner membrane*	*Outer membrane*	*Total*	*Soluble*	*Membrane*
Cytochrome oxidase	2050	4500	600	N.D.	—	—
Monoamine oxidase	6	3	38	N.D.	—	—
Acid phosphatase	23	N.D.	169	1039	678	1932
NADPH–cytochrome *c* reductase	4	3	30	4	5	7
5′-Nucleotidase*						
pH 5.5	7.6			715	440	1410
pH 7.5	7	10	140	563	341	1312
pH 9.0	4.3			100	70	440
Galactosidase	—	—	—	—	—	—
Galactosyltransferase	—	—	—	—	—	—

Marker enzyme	*S-fraction*			*L-fraction*	*Microsomes*	*Plasma membrane*
	Total	*Soluble*	*Particulate*			
Cytochrome oxidase	60	—	—	206	39	—
Monoamine oxidase	1	—	—	N.D.	N.D.	1
Acid phosphatase	694	736	482	220	51	35
NADPH–cytochrome *c* reductase	19	11	—	40	94	14
5′-Nucleotidase*						
pH 5.5	330	427	241			
pH 7.5	299	370	—	118	58	
pH 9.0	70	44	78			1428
Galactosidase	—	—	—	—	—	2.6
Galactosyltransferase	—	—	—	—	—	0.5

* *cf.* also Fig. 1.

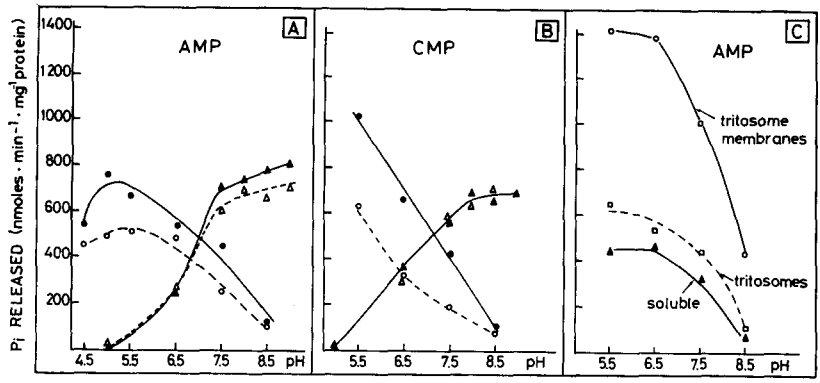

Fig. 2. pH–activity curve of 5′-nucleotidase in tritosomes and plasma membrane. Incubation medium: 8 mM $MgCl_2$, 100 mM KCl, 50 mM Tris–HCl buffer, pH 7.5–9 and 50 mM citrate buffer from pH 4.5 to 6.5, 4 mM AMP or CMP. Final volume 1 ml. Incubation 20 min at 37 °C. Amounts of protein used: plasma membrane 100 μg, whole tritosomes 50 μg, soluble tritosome fraction 100 μg, dialyzed tritosome membrane 50 μg. ●—●, tritosomes; ○—○, tritosomes + 20 mM L(+)-tartrate; ▲—▲, plasma membrane; △—△, plasma membrane + 20 mM L(+)-tartrate.

The inner mitochondrial membrane indicated in Table I is the fraction obtained from inner membrane + matrix particles (*cf.* Methods and ref. 25). The plasma membrane and the lysosomes contain 5′-nucleotidases that Kaulen *et al.*[46] could discriminate on the basis of K_m values, pH optima and sensitivity to L(+)-tartrate. We confirm the main conclusions of these authors with some qualifications (Fig. 2): L(+)-tartrate inhibited no more than 50% of the lysosomal CMPase activity and 30% of the AMPase activity measured at pH 6.5; we found the pH optima to be around 5 for lysosomes and 9 for plasma membrane. Similarly to acid phosphatase (ref. 47 and Table I) about 50% of the total lysosomal 5′-nucleotidase is soluble but the highest specific activity is found in the membrane (Fig. 2C). From marker-enzyme activities (Table I) the tritosome and S-fraction represent better lysosome preparations than the L-fraction; their soluble components have comparable acid phosphatase and 5′-nucleotidase activities.

Comments on phospholipase A assay

The assessment of phospholipase A activities in subcellular fractions presents several pitfalls partly due to the insolubility of substrates in water. (1) Substrates therefore must be emulsified. As shown recently by Hauser[48] emulsification by sonication is accompanied by a more or less pronounced formation of lysoderivatives which can be inhibitors of phospholipase A (ref. 49 and unpublished results). The emulsions used in our tests were prepared in 5–10 ml of water with 5% ethyl ether and checked for their content in lysoderivatives and when more than 5% of lysoderivatives were found they were discarded. In addition it is difficult to obtain homogeneous concentrated phosphatidylethanolamine suspensions by sonication, without detergent. The concentrations indicated in tables and figures correspond to that of phosphatidylethanolamine actually in emulsion (it sometimes happened to be only of the order of some high K_m values). (2) Because of the difficulty in obtaining reproducible micellar suspensions and hence protein lipid-interactions, the K_m values and the activities given have only a relative significance (*cf.* ref. 50). They nevertheless permit valid

comparisons since the same micellar suspension was used to test different membrane preparations as for instance inner and outer mitochondrial membranes. Generally, all experiments involved a cross-checking of phospholipase assays in at least two different subcellular organelles. (3) Phospholipase A activity was determined by measuring either the formation of free fatty acids from both endogenous or exogenous phospholipids or the accumulation of lyso[^{32}P]phosphatidylethanolamine from [^{32}P]-phosphatidylethanolamine. The nature of the free fatty acids released was taken as indicative of the specificity of the phospholipase A assigning to A_1 the release of saturated fatty acids and to A_2 the release of unsaturated fatty acids. An accumulation of lysoderivatives may account for a phospholipase A activity only in the absence of lysophospholipase since in this case lysophosphatides are transformed into water-soluble compounds. In our tests, the sum of radioactivity of [^{32}P]phosphatidylethanolamine + lyso[^{32}P]phosphatidylethanolamine remained constant within 10% and there was no significant formation of labelled glycero[^{32}P]phosphorylethanolamine in the water phase. Comparative assays of activities of various subcellular membranes were carried out under conditions giving less than 20% hydrolysis of the exogenous substrate. Under these conditions, the phospholipid hydrolysis was linear with the enzyme concentration (Fig. 3) and the time of incubation up to 40–60 min. (4) Phospholipase A activities were determined at acid pH (4.5) and at alkaline pH (8–9).

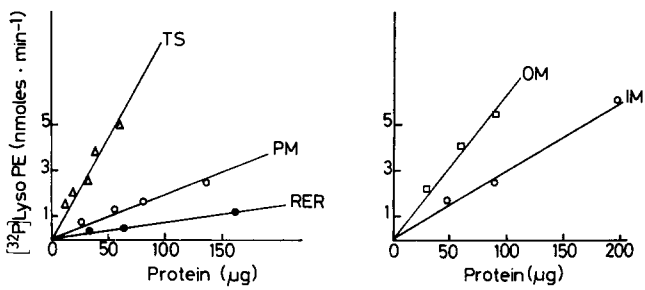

Fig. 3. Effect of protein concentration on lyso-[^{32}P]phosphatidylethanolamine ([^{32}P]Lyso-PE) accumulation. Left: 150 μM [^{32}P]phosphatidylethanolamine, 15 min, 37 °C. Rough endoplasmic reticulum (RER) pH 8.2. Plasma membrane (PM), pH 9.0. Tritosome soluble (TS), pH 4.5. Right: 200 μM [^{32}P]phosphatidylethanolamine, 15 min, 37 °C, pH 9.0. Outer (OM) and inner (IM) mitochondrial membranes.

$CaCl_2$ and KCl were found to inhibit acid phospholipase A activity (in agreement with ref. 51) but to activate the alkaline phospholipases A to different extents (Table II). Among the alkaline phospholipases, the microsomal one was the less sensitive to the presence of Ca^{2+} and K^+. $CaCl_2$ had an optimal stimulatory effect around 2–4 mM. Variations in $CaCl_2$ concentrations up to 10 mM did not modify the nature of the fatty acids released. In most, if not all of the tests presented here, $CaCl_2$ and KCl were added at alkaline pH and omitted at acidic pH. (5) No lytic agent was used for assaying the activity of membrane-bound phospholipases. Inner mitochondrial membrane devoid of matrix, outer mitochondrial membrane, lysosomal and plasma membranes, rough reticulum were used as such. Additions of detergent in the reaction medium to unmask possible cryptic activity may actually result in uncontrollable conditions of test. For instance, as described recently[52] adding Triton X-100 at 0.5 mg/ml to

TABLE II

EFFECT OF $CaCl_2$ AND KCl ON RAT LIVER PHOSPHOLIPASE A ACTIVITIES

2 mM $CaCl_2$ and/or 0.1 M KCl; 160 μM [^{32}P]phosphatidylethanolamine was used with mitochondria (0.15 mg protein), microsomes (0.1 mg protein) and plasma membrane (0.090 mg protein) and 200 μM with S-fraction (0.12 mg protein). Incubation 15 min at 37 °C. N.D., not detected.

	pH	*Lyso*[^{32}P]*phosphatidylethanolamine formed* (*nmoles·mg^{-1} of protein*)			
		—	*$CaCl_2$*	*KCl*	*$CaCl_2$ + KCl*
Mitochondria	9	N.D.	8	18	23
Microsomes	8.5	13	21	53	49
Plasma membrane	8.5	N.D.	42	38	65
S-fraction	4.5	148	73	54	30
	8.5	6	6	9	9

Escherichia coli phospholipase A_1 decreases by 40-fold the affinity of the enzyme for its substrate.

Characterization of phospholipase A activities in different subcellular fractions

The pH optimum is an easy criterion for a preliminary discrimination of subcellular phospholipases A. Nevertheless there are discrepancies between the optimal pH values reported by different authors for plasma membrane[21–23], lysosomal[12,15,16,18,19], microsomal[11,14] and mitochondrial[9,10,12,49] phospholipases A. As shown in Fig. 4, the optimal pH of phospholipase A present in mitochondrial and plasma membrane fractions is above 9, that of microsomes is around 8.5. In tritosome fractions, the alkaline phospholipase activity (optimal pH around 9) is ten times lower than the

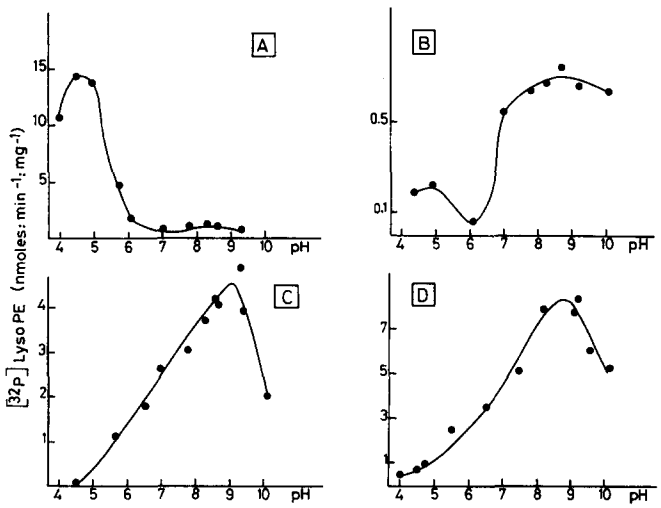

Fig. 4. pH–activity curves for phospholipases A in different rat liver subcellular fractions. Incubation conditions as described in Methods. 15 min at 37 °C. A. Tritosomes, 0.32 mg protein and 160 μM [^{32}P]phosphatidylethanolamine. B. Mitochondria, 1 mg protein and 117 μM [^{32}P]phosphatidylethanolamine; C. Plasma membrane, 0.15 mg protein and 150 μM [^{32}P]phosphatidylethanolamine; D. Rough endoplasmic reticulum, 0.077 mg protein and 215 μM [^{32}P]phosphatidylethanolamine.

TABLE III

PHOSPHOLIPASE A ACTIVITIES IN SUBCELLULAR MEMBRANE FRACTIONS

Incubation conditions as described in Methods. Acid phospholipase measured at pH 4.5 alkaline phospholipase at pH 9.0. Values expressed as nmoles of lyso-[^{32}P]phosphatidylethanolamine formed·min^{-1}·mg^{-1} protein. In parentheses: number of experiments.

	Mitochondria (3)			*Tritosomes* (2)		
	M_D	*Inner membrane*	*Outer membrane*	*Total*	*Soluble*	*Membranes*
Acid phospholipase	0.3	0.9	2.1	50	90	4.5
Alkaline phospholipase	1.4	3.0	4.8	2.5	0.8	5.2
Ratio: acid phospholipase / alkaline phospholipase	0.2	0.3	0.2	20	112	0.9

	S-fraction (2)			*Microsomes* (3)		*Plasma membrane* (3)
	Total	*Soluble*	*Particulate*	*Total*	*Rough endoplasmic reticulum*	
Acid phospholipase	23	26.4	3.3	<0.2	0.7	<0.1
Alkaline phospholipase	3.7	11.4	6.9	3.3	8.1	6.4
Ratio: acid phospholipase / alkaline phospholipase	6.2	2.6	0.5	0.06	0.08	0.01

acid one whose optimal pH is around 4.5. The fact that all the rat liver alkaline phospholipase A activities have their optimal pH above 8 and that most of them are membrane-bound (Table III), points to the necessity of a careful evaluation of cross contaminations between subcellular membrane fractions.

Lysosomal phospholipases A. In all the lysosomal fractions tested, the acid phospholipase A activity (pH 4.5) was always several fold higher than the alkaline one (pH 8–9). In order to localize the acid and alkaline phospholipase A activities within lysosomes, tritosomes were fractionated into their soluble and particulate components as shown in Fig. 1, and the distribution of phospholipase activities compared to the distribution of acid phosphatase and to 5′-nucleotidase and NADPH–cytochrome *c* reductase marker enzymes for possible contaminants. The phospholipase A activity measured at pH 4.5 was mainly soluble, while that measured at pH 8.5 was essentially membrane-bound (Table III).

The acid, soluble phospholipase activity exhibits a $A_1 + A_2$ specificity. This could be the result of the sequential action of a phospholipase and a lysophospholipase or a conjoined action of phospholipases A_1 and A_2. The latter alternative is more likely since lysophosphatides accumulated (Fig. 5). The phospholipase A_2 appeared to be less active than A_1. Since Stoffel and Trabert[17] have shown that Triton WR-1339 inhibits the acid lysosomal soluble phospholipase A_2, a possible effect of Triton was evaluated by using the S-fraction (Fig. 1) as another source of soluble lysosomal

enzymes. From the same incubation test tube, analysis of the saturated and unsaturated fatty acids showed that the acid phospholipase A_1 (K_m = 770 μM, V = 40 nmoles·min^{-1}·mg^{-1} protein) was about twice more active than the A_2 (K_m = 680 μM, V = 20 nmoles·min^{-1}·mg^{-1} protein) and had slightly less affinity than phospholipase A_2.

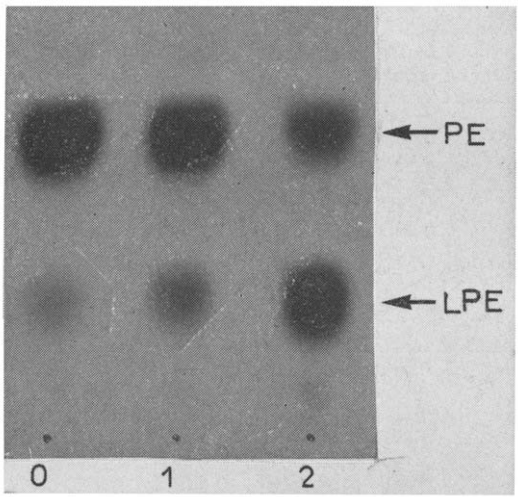

Fig. 5. Formation of lyso[^{32}P]phosphatidylethanolamine (LPE) by incubation of [^{32}P]phosphatidylethanolamine (PE) with tritosomes. Autoradiogram of a Merck silicic acid chromatoplate. Incubation conditions, extraction and separation as described in Methods. 0, zero time; 1, 20 min of incubation at pH 8.5; 2, 20 min of incubation at pH 4.5.

The alkaline membrane-bound phospholipase in lysosomes (Table III) has an apparent K_m of 120 μM and V of 5.5 nmoles·min^{-1}·mg^{-1} (Fig. 6). The comparatively low activity of the membrane-bound phospholipase contrasts with the considerable activities of the acid phosphatase in the same preparation (Table I). The tritosome membranes used here (Fig. 6) had been extensively dialyzed, when tritosomes are disrupted by short hypotonic treatment and centrifuged immediately more acid phospholipase remains bound to the membrane[7]. A comparative evaluation of these activities in a membrane preparation endowed with an alkaline phospholipase may therefore be useful to decide whether the tested phospholipase is of lysosomal origin.

Microsomal phospholipase A. This was studied in the rough endoplasmic reticulum which is less contaminated by other subcellular membranes than the smooth reticulum[25]. Its pH optimum was around 8.5 (Fig. 4) and its specificity A_1 (Table IV). Microsomal phospholipase A_1 hydrolyzed added [^{32}P]phosphatidylethanolamine preferentially to endogenous phospholipids. Values of the apparent K_m for phosphatidylethanolamine varied from 200 to 400 μM (Fig. 6B). As shown in Table II the microsomal phospholipase A was slightly stimulated by $CaCl_2$ but more so by KCl which extracts the enzyme from the membrane. A stimulating effect of KCl was still observed when using a solubilized preparation of microsomal phospholipase A.

Delipidation of microsomes did not inactivate the phospholipase A. Specific activities of 3.9 and 6 nmoles lysophosphatidylethanolamine·min^{-1}·mg^{-1} protein were found before and after delipidation respectively. The phospholipase A of delip-

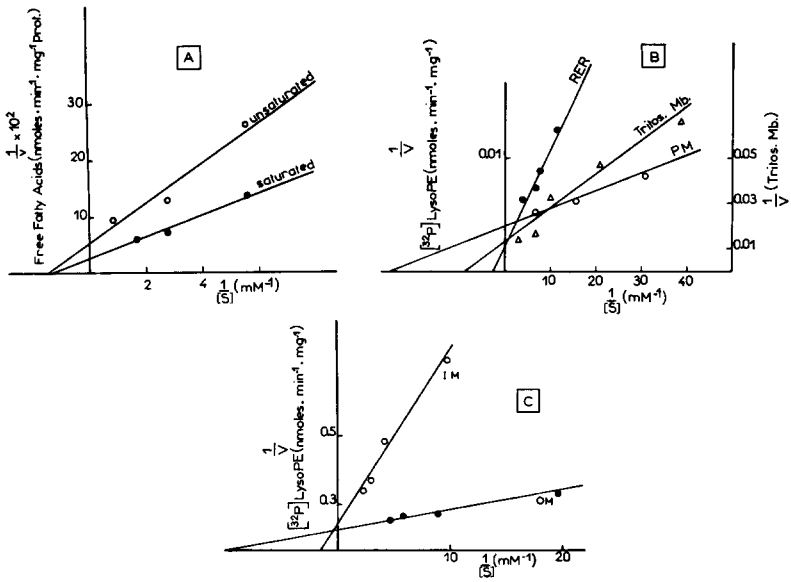

Fig. 6. Effect of phosphatidylethanolamine concentration on phospholipase A activities. A. Saturated and unsaturated fatty acid release by S-fraction at pH 4.5, 160 μg protein, 30 min at 37 °C. Phospholipase A_1: K_m = 770 μM, V = 40 nmoles·min^{-1}·mg^{-1}. Phospholipase A_2: K_m = 680 μM, V = 20 nmoles·min^{-1}·mg^{-1}. B. Lyso[^{32}P]phosphatidylethanolamine ([^{32}P]LysoPE) accumulation with rough endoplasmic reticulum (RER), plasma membrane (PM) and membranes of dialyzed tritosomes (Tritos. Mb.). 50–100 μg protein, 15 min at 37 °C, pH 8.2 (RER) or 9 (PM). K_m = 390 μM (RER), 40 μM (PM) and 120 μM (Tritos. Mb.). V = 22 (RER), 24 (PM) and 5.5 (Tritos. Mb.) nmoles·min^{-1}·mg^{-1}. C. As in B with inner (IM) and outer (OM) mitochondrial membranes at pH 9. K_m = 400 μM (IM) and 90 μM (OM), V = 10.4 (IM) and 10 nmoles·min^{-1}·mg^{-1} (OM).

TABLE IV

NATURE OF THE FATTY ACIDS RELEASED FROM EXOGENOUS [^{32}P]PHOSPHATIDYLETHANOLAMINE BY THE ALKALINE PHOSPHOLIPASES A CONTAINED IN RAT LIVER SUBCELLULAR FRACTIONS

0.1 to 0.4 mg of protein was used with 215 μM [^{32}P]phosphatidylethanolamine in a total standard volume of 2 ml. Incubation 30 min at 37 °C. Percentages were evaluated from peak areas.

Fractions	*pH*	*% Saturated fatty acids*	*% Arachidonic acid*	*Ratio* $\frac{unsaturated}{saturated}$
Lysosomal membrane	9.0	33.2	42	2.0
Microsomes	8.3	69	15	0.4
Plasma membrane	9.0	85.9	N.D.	0.2
Mitochondria$_D$	9.0	24.9	40	3.0
Inner membrane	9.0	24.6	41	3.1
Outer membrane	9.0	27.0	35	2.7
Snake venom	8.3	3.1	55	28.4
NaOH		45.6	21	1.2

idated microsomes was easily extractable by 0.3 M KCl and the soluble extract had a specific activity three times higher than that of the original material.

Plasma membrane phospholipase A. It has been shown in Fig. 4 that the phospholipase A of plasma membrane has a pH optimum around 9. According to Table IV, using [^{32}P]phosphatidylethanolamine as an exogenous substrate, up to 86% of the

fatty acids released were found to be saturated. However, as a special case among organelle membranes, plasma membrane phospholipids are mainly saturated[25,53] and an endogenous phospholipid hydrolysis predominating over that of the added phosphatidylethanolamine could explain the preferential release of saturated fatty acids in our test conditions. We have therefore compared the fatty acid release in the absence and in the presence of exogenous substrate. In the absence of exogenous substrate the release of fatty acids during the first minutes of incubation was 25% of that found in the presence of substrate; it levelled out after 10 min while, in the presence of exogenous substrate, it remained linear up to at least 1 h. On the other hand there was virtually a one to one stoichiometric relationship between the amount of lyso[^{32}P]phosphatidylethanolamine accumulated and that of fatty acid released, confirming that in plasma membrane endogenous phospholipids were not hydrolyzed preferentially to the added [^{32}P]phosphatidylethanolamine. Based on these data, it may be concluded that the specificity of the plasma membrane phospholipase A is essentially of A_1 type. A K_m of 40 μM (Fig. 6) was found for phosphatidylethanolamine, a value close to 30 μM found by Newkirk and Waite[21].

In conclusion, plasma membrane contains a phospholipase A which is membrane-bound, has a pH optimum around 9, a predominant A_1 specificity, is stimulated by $CaCl_2$ (up to 4 mM) and by KCl; the Ca^{2+} and K^+ activations of the plasma membrane phospholipase A are apparently additive (Table II), in contrast with Ca^{2+} and K^+ activations in other subcellular membranes.

Mitochondrial phospholipase A. After a "slight digitonin treatment" of a mitochondrial preparation (Fig. 1 and ref. 25) 80% of lysosomal and plasma membrane contaminants and 40% of microsomal contaminants were removed but more than 80% of the alkaline phospholipase A activity remained in the purified mitochondrial preparation (M_D). This remaining activity had a predominant A_2 specificity in contrast with the A_1 specificity found in microsomes and plasma membrane (Table III).

Supporting evidence for a distinct phospholipase activity in mitochondria was found in the character of a remarkable preference of the mitochondrial enzyme for endogenous substrates. This conclusion is based upon the following: while the amount of lyso[^{32}P]phosphatidylethanolamine formed by incubation with plasma membrane and microsomes (14 and 29 nmoles·mg^{-1} protein, respectively) was virtually equal to the amount of fatty acids released (12 nmoles for plasma membrane and 33 for microsomes) the amount of fatty acids released in the presence of mitochondria is five times as high as the amount of lyso[^{32}P]phosphatidylethanolamine accumulated. Since no appreciable radioactivity was detected in the water phase, it is concluded that the increased fatty acid release by mitochondria is not due to the presence of a lysophospholipase. Nor can it be attributed to lipase activity, which was not detectable with triolein as substrate and amounted to only 8 nmoles fatty acid released per min per mg protein with mono-olein (no activity being detectable in the absence of added monoglyceride). Thus, the free fatty acids found on incubation of mitochondria can arise only from endogenous phospholipid and indeed we[5] and Bjørnstad[10] have shown that the release of free fatty acid equals the amount of lysophospholipid formed.

For a better understanding of the role of a phospholipase A_2 within the mitochondrion, the distribution of its activity between inner and outer membrane was measured together with that of marker enzymes. In previous reports[5–7] the outer membrane was found to exhibit the highest phospholipase A_2 specific activity, al-

though a significant amount of phospholipase A_2 was still linked to the inner membrane + matrix fraction. Since, by the method of Parsons some outer membrane remains in the inner membrane + matrix fraction, the distribution of the phospholipase A was reinvestigated in membrane fractions obtained by digitonin treatment[26]. The results are presented in Fig. 7. A standard mitochondria preparation was fractionated into: inner membrane + matrix particles (I + Ma), outer membrane (O),

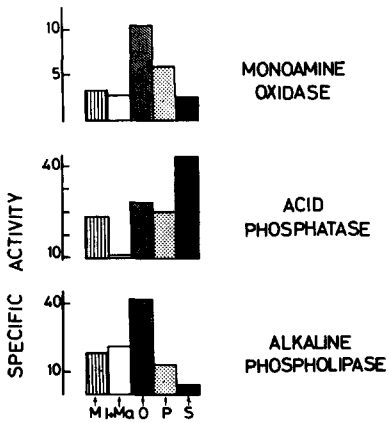

Fig. 7. Submitochondrial distribution of alkaline phospholipase A activities ($A_1 + A_2$) in relation to marker enzymes. Fractionation of rat liver mitochondria according to Schnaitman and Greenawalt[26]. The fractions are represented along the abscissa in the order in which they are isolated: M, mitochondria; I + Ma, inner membrane + matrix particles; O, outer membrane; P, intermediate fraction; S, supernatant. Phospholipase A activity assessed by the release of fatty acids at pH 8.2 and expressed in nmoles fatty acid·mg^{-1} protein. 250 μM phosphatidylethanolamine, 1.8 to 3 mg protein, 30-min incubation at 37 °C.

an intermediate fraction (P) and a soluble fraction (S). The soluble fraction showed the highest acid phosphatase and acid phospholipase activities. The inner membrane + matrix fraction apparently devoid of lysosomal contaminants but still containing some monoamine oxidase exhibited a significant alkaline phospholipase activity comparatively higher than that which could be predicted if it were only due to contaminant outer membrane. To further investigate this point, another experiment was carried out using a preparation of inner membrane vesicles[25]. As shown in Table III the specific activity of phospholipase A in isolated inner mitochondrial membrane, after most of the matrix proteins had been removed and when tested in the same protein concentration range as outer membrane, is nearly as high as that of the outer membrane. It is therefore clear that both the outer and the inner membrane are endowed with a phospholipase A_2 activity, a finding in agreement with ref. 13. The question as to whether the same type of phospholipase A_2 is distributed in the outer and the inner mitochondrial membrane cannot be decided as yet; the finding of different K_m values for phosphatidylethanolamine in the outer (90 μM) and inner (400 μM) mitochondrial membranes (Fig. 6C) may be due for instance to differences in the electrical properties of the two membranes[54]. The demonstration of a phospholipase A_2 in inner mitochondrial membrane preparations is of the highest interest in view of their high degree of purity; indeed it represents, so far, the least ambiguous proof of the presence of a phospholipase A_2 in mitochondria.

This comparative survey of phospholipases in subcellular fractions of the rat hepatocyte qualified by an appraisal of the extent of cross contaminations shows that membranes of all subcellular organelles are endowed with phospholipase A activities. However, the exact level of their respective activities is difficult to assess due to many interferences. Among, them, acylation processes may well mask a phospholipase A activity. This question will now be examined.

Masking of phospholipase A activity by reacylation

An acylation process is detected as incorporation of labelled fatty acids into endogenous phospholipids provided that a *in situ* phospholipase catalyzes the formation of lysophosphatides. In this respect the behaviour of phosphatidylcholine and phosphatidylethanolamine differs from that of phosphatidic acid.

Acylation of lysophosphatidylcholine and lysophosphatidylethanolamine by microsomes and plasma membrane is already known[55–57]. The presence of a lysophospholipid reacylating system in the outer mitochondrial membrane has been shown in a previous paper to be accounted for by contaminant microsomes[7]. These results have been extended to the inner membrane + matrix particles isolated after removal of the outer mitochondrial membrane by hypotonic swelling[27] and collected as low-speed pellet. Also, in this case the labelling of phosphatidylethanolamine and phosphatidylcholine appears to be due to contaminant microsomes (Fig. 8). These results are in agreement with those of McMurray and Dawson[56] and Eibl *et al.*[55] who found that the small reacylating activity in the whole mitochondria was due to microsomes present in their preparation.

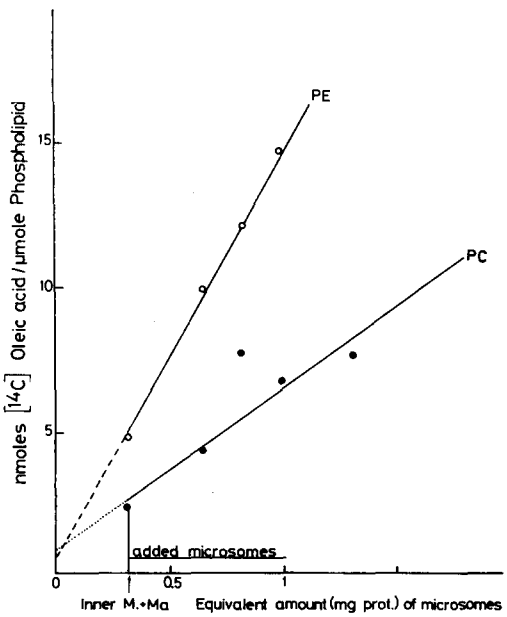

Fig. 8. Incorporation of [^{14}C]oleic acid into membrane phosphatidylethanolamine (PE) and phosphatidylcholine (PC) of an inner membrane + matrix preparation, as a function of microsomal contamination. Incubation conditions: 2 mM ATP, 0.4 mM CoA, 1.25 mM $MgCl_2$, 0.25 mM $CaCl_2$, 50 mM NaF, 0.6 mM [^{14}C]oleic acid, 75 mM Tris–HCl buffer, pH 8, 20 min at 37 °C, 6.3 mg of protein in 4 ml final volume.

However, a reacylating capacity of mitochondria could be revealed when [^{14}C]oleic acid incorporation into phosphatidic acid was studied. A significant incorporation of [^{14}C]oleic acid, not accounted for by microsome contamination, was detected in both phosphatidic and lysophosphatidic acids in isolated outer mitochondrial membrane preparations; lysophosphatidic acid was the main product, the amounts of the two labelled derivatives being independent of the presence of glycerol 3-phosphate (Fig. 9A). In the same test conditions no labelling was catalyzed by inner membrane + matrix particles or by plasma membrane[58]. The microsomal system, very active in the presence of added glycerol 3-phosphate (20–50 times more than outer membrane) and present both in rough and smooth endoplasmic membranes[58], produced mainly phosphatidic acid (Fig. 9B). This difference in the labelling of phosphatidic and lysophosphatidic acids by microsome and mitochondria and the localization in the outer membrane of the mitochondrial system has also been reported by Daae and Bremer[59]. Nevertheless as also remarked by those authors[59] the accumulation of lysophosphatidic acid (Fig. 9) does not seem to be due to the action of phospholipase A but rather lysophosphatidic acid would be an intermediate in the formation of phosphatidic acid.

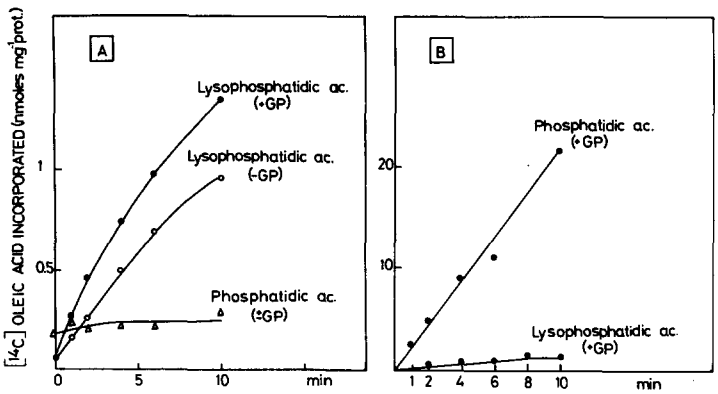

Fig. 9. Incorporation of [^{14}C]oleic acid into phosphatidic and lysophosphatidic acids by an outer mitochondrial membrane (A) and a microsomal (B) preparation. The incubation medium contained 5 mM $MgCl_2$, 20 mM Tris–HCl, pH 7.8, 25 mM NaF, 0.4 mM CoA, 2.5 mM α-glycerophosphate (GP), 0.4 mM [^{14}C]oleic acid, 0.4 mM ATP, pH 7.8, and outer mitochondrial membrane (3 mg protein) or microsomes (4.8 mg protein) in a final volume of 2 ml.

In order to check an action of phospholipase A on endogenous phosphatidic acid, the following experiment was performed. Phosphatidic acid and its lyso-derivative were labelled *in situ* by preincubation of mitochondria with [^{32}P]ATP. Then either $CaCl_2$ or CoA + oleic acid were added. A parallel experiment was carried out with microsomes. As shown in Table V, Ca^{2+} which is an effector of phospholipase A in mitochondria and microsomes, does not enhance the formation of lysophosphatidic acid. This result suggests that phospholipase A does not attack endogenous phosphatidic acid and that in consequence the accumulation of [^{14}C]oleyl-glycerol 3-phosphate observed in the above-mentioned experiments (Fig. 9) is more the expression of synthetic systems than that of degradative ones in mitochondria. It may be noted incidently that the outer mitochondrial membrane seems to be responsible for the above mentioned accumulation of ^{32}P-labelled phosphatidic and lysophosphatidic

TABLE V

FORMATION OF ^{32}P-LABELLED PHOSPHATIDIC AND LYSOPHOSPHATIDIC ACIDS IN RAT LIVER MITOCHONDRIA AND MICROSOMES IN THE PRESENCE OF [γ-^{32}P]ATP. EFFECT OF Ca^{2+}

The basic incubation medium was made of 10 mM sodium glutamate, 30 mM NaF, 0.25 mM $MgCl_2$, 20 mM Tris–maleate buffer, pH 7.8, 0.4 mM [γ-^{32}P]ATP, pH 7.8 (3200 dpm/nmole) and 6 mg of protein in a final volume of one ml; as indicated, 2 mM $CaCl_2$ or 0.5 mM CoA + 1 μmole oleic acid were added.

	Incubation period (min)	*Additions*	*Phosphatidic acid*	*Lysophosphatidic acid*
			(*pmoles·mg^{-1} protein*)	
Mitochondria	10	—	231	45
	20	—	465	30
	10	Ca^{2+}	36	4
	10* + 10	Ca^{2+}	251	29
	10	CoA + oleic acid	25	N.D.
Microsomes	10	—	150	75
	20	—	310	46
	10	Ca^{2+}	76	10
	10* + 10	Ca^{2+}	129	28

* 10 min preincubation prior to Ca^{2+} addition.

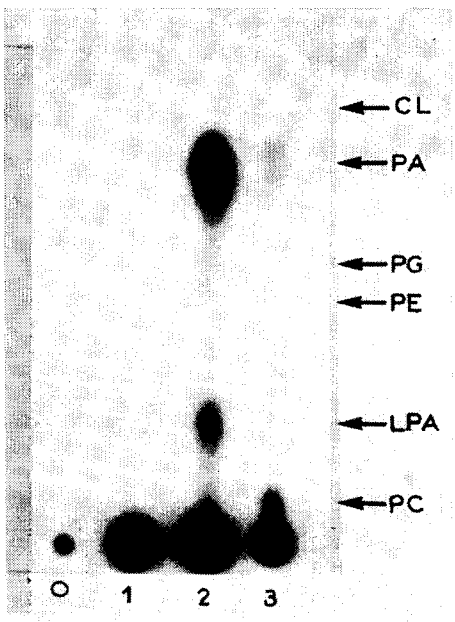

Fig. 10. Incorporation of ^{32}P from [γ-^{32}P]ATP into the phospholipids of inner membrane + matrix particles (1), outer mitochondrial membrane (2) and microsomes (3). 0 = zero time. Incubation medium: 10 mM glutamate, 15 mM NaF, 20 mM Tris–HCl buffer, pH 7.7, 0.4 mM [γ-^{32}P]ATP 4 to 6 mg of protein. Incubation 10 min at 37 °C. Autoradiogram of a Merck silicic acid chromatoplate after two successive upward migrations: first in acetone–light petroleum (1:3, v/v) and second in chloroform–methanol–acetic acid–water (80:13:8:0.3, by vol.). The lecithin spot revealed by the Dragendorff reagent (*cf.* ref. 44) was, as indicated, slightly above the starting line and practically not labelled. The radioactive spot remaining at the origin has not been identified. Abbreviations: PA, phosphatidic acid; PE, phosphatidylethanolamine; LPA, lysophosphatidic acid; PC, phosphatidylcholine; PG, phosphatidylglycerol; CL, cardiolipin.

acids in mitochondria and even appears more efficient than microsomes in this process as illustrated by autoradiography of a thin-layer chromatoplate (Fig. 10).

DISCUSSION

This paper, directed towards a comparative study of subcellular phospholipase A species, follows a preceding one where the isolation and characterization of the subcellular fractions used were extensively described[25].

Our results on lysosomal phospholipase A activities having a pH optimum at 4.5, agree with those already published on the subject[15–19]. Using short incubation periods (15 to 30 min compared to hours in ref. 18) we did not observe such an extensive degradation of the phospholipid molecules as those described by Fowler and de Duve[18] but, like those authors we could detect (*cf.* also ref. 7) an accumulation of lysoderivatives when using whole tritosomes. This can be explained by the fact, also reported by refs. 17 and 19, that the soluble fraction of lysosomes contains two acid phospholipases (A_1 and A_2).

Two alkaline phospholipase A activities are present concomitantly in the membranes tested: a A_2 specificity was found to be predominant in mitochondria and lysosomal membranes and a A_1 specificity in microsomes and plasma membrane. These findings are in agreement with refs. 4–8 and 11–13 for mitochondria, ref. 51 for lysosomal membrane, refs. 11 and 23 for microsomes, and ref. 21 for plasma membrane. On the other hand, Victoria *et al.*[22] and Van Golde *et al.*[23] concluded that phospholipase A_2 predominates in plasma membrane.

The mitochondrial phospholipase A has already been the subject of several recent publications (refs. 4–7, 12, 19 and 49). Nevertheless its specific activity being among the smallest of those measured in rat liver subcellular membrane fractions and because of the great difficulty in avoiding contamination it was of interest to bring new experimental evidence confirming the existence of a phospholipase A of mitochondrial origin. The best proof of the existence of a mitochondrial phospholipase A is the detection of phospholipase A_2 in inner membrane vesicles with a specific activity comparable to that of isolated outer membrane. The several mechanical treatments involved in the preparation and isolation of inner membrane vesicles with a very low matrix protein content may be responsible for an unmasking of the phospholipase A_2 activity in this membrane, an activity which cannot be attributed to membrane contaminants since it has been shown[25] that these mitochondrial membrane preparations, present a high degree of purity. So, an exclusive localisation of phospholipase A_2 in outer mitochondrial membrane cannot be envisaged. An attractive hypothesis would be that this enzyme is near or at junction points[60] between the two mitochondrial membranes.

As discussed above (Table IV) A_1 specificity found in plasma membrane and A_2 specificity in mitochondria has been one of the criteria used in this study to distinguish between the two. The fact, already observed[5,10] that mitochondrial phospholipase A hydrolyzes preferentially endogenous phospholipids when no Triton X-100 is present in the incubation medium was another criterion; it has been related to the control of mitochondrial swelling[61].

A preferential release of arachidonic acid by mitochondrial phospholipase A was observed. This may be due either to a preferential hydrolysis of endogenous

phosphatidylethanolamine which contains more arachidonic acid than lecithin does[25] or to a less tight packing of the highly unsaturated fatty acids, as illustrated by the fact that phospholipid films are more expanded when the number of double bonds is greater[62], rendering the arachidonoyl ester bond more accessible to the enzyme. A shortening of the carbon chain of the saturated fatty acids having an effect similar to a high unsaturation, microsomal and plasma phospholipases A_1 release preferentially palmitic acid. On the other hand, solubilized mitochondrial phospholipase A_2 has been shown[49] to release oleic acid preferentially to arachidonic acid from rat liver phosphatidylethanolamine: this may be due to different protein–micelle interactions with the soluble enzyme.

Since the lysophosphatides produced by phospholipase A have a lytic effect on membranes one may wonder which mechanism operates at the membrane level to control the *in situ* activity of a given phospholipase A. Reacylation of lysophosphatides is apparently one of the possible mechanisms as found especially in the endoplasmic reticulum. However, mitochondria were not found to incorporate actively labelled fatty acids into their major phospholipids. In these organelles, removal of lysophosphatides might occur by exchange with microsomal phosphatides through a mechanism essentially similar to the mitochondrial–microsomal phospholipid exchange discovered by Wirtz and Zilversmit[63]. One may equally suspect the presence, in the mitochondrial membrane, of a phospholipase A inhibitor.

Mention is made of the ability of mitochondria to acylate glycerophosphate by a system localized in the outer membrane, as also observed by others[64–67]. The accumulation of lysophosphatidic acid is apparently not due to the action of phospholipase A but rather may result from a lack of lysophosphatidic acid acylating system shown, by Lands and Hart[68], to be exclusively localized in microsomes. Results of Daae and Bremer[59] indicated that microsomes may cooperate with the outer membrane to further acylate mitochondrial lysophosphatidic acid. One may extend to other cytoplasmic systems this concept for a possible degradation of lysophosphatidic acid into glycerides. Since the labelled lysophosphatidic acid came, in mitochondria, from endogenous precursors, if this labelling were connected to glyceride turnover, it could provide a means of buffering the concentration of free fatty acids available to the inner membrane + matrix compartment.

Assignment of a specific role to phospholipase A in cell physiology is still speculative. However, it is reasonable to postulate that phospholipase A by inducing localized micellar reorganization of the intramembrane lipids[69,70] favors the formation of fusion areas[71] between subcellular membranes within a cell, and between the plasma membranes of adjacent cells and thereby increases exchange of material between membranes.

ACKNOWLEDGEMENTS

For some parts of the work the authors gratefully acknowledge the technical assistance of Mr R. Césarini (fatty acid analysis) and Mrs J. Chabert.

This investigation was supported by research grants from the C.N.R.S. (E.R.A. No. 36), from the "Fondation pour la Recherche Médicale", and the D.G.R.S.T.

This work represents partial fulfillment of a thesis work (J.N.) to be submitted to the Grenoble University of Sciences and Medicine.

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