Hydrolysis of Membrane Phospholipids by Phospholipases of Rat Liver Lysosomes

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(1) The hydrolysis of ^{32}P - or *myo*-[2- ^3H]inositol-labelled rat liver microsomal phospholipids by rat liver lysosomal enzymes has been studied. (2) The relative rates of hydrolysis of phospholipids at pH 4.5 are: sphingomyelin > phosphatidylethanolamine > phosphatidylcholine > phosphatidylinositol. (3) The predominant products of phosphatidylcholine and phosphatidylethanolamine hydrolysis are their corresponding lyso-compounds, indicating a slow rate of total deacylation. (4) Ca^{2+} inhibits the hydrolysis of all phospholipids, though only appreciably at high (>5 mM) concentration. The hydrolysis of sphingomyelin is considerably less sensitive to Ca^{2+} than that of glycerophospholipids. (5) Analysis of the water-soluble products of phosphatidylinositol hydrolysis (by using *myo*-[^3H]inositol-labelled microsomal fraction as a substrate) produced evidence that more than 95% of the product is phosphoinositol, which was derived by direct cleavage from phosphatidylinositol, rather than by hydrolysis of glycerophosphoinositol. (6) This production of phosphoinositol, allied with negligible lysophosphatidylinositol formation and a detectable accumulation of diacylglycerol, indicates that lysosomes hydrolyse membrane phosphatidylinositol almost exclusively in a phospholipase C-like manner. (7) Comparisons are drawn between the hydrolysis by lysosomal enzymes of membrane substrates and that of pure phospholipid substrates, and also the possible role of phosphatidylinositol-specific lysosomal phospholipase C in cellular phosphatidylinositol catabolism is discussed.

Lysosomes of many tissues have been shown to contain phospholipases (for reviews see Barrett & Heath, 1977; Waite *et al.*, 1976), though the relative importance of these phospholipases in cellular phospholipid metabolism is still unclear. The study of the lysosomal phospholipases has, like that of most phospholipases, been largely confined to pure phospholipid substrates rather than their likely substrates *in vivo*, namely biological membranes. It is evident in many cases that only by using membrane substrates is a true picture of the activity and specificity of phospholipases obtained (for example, see Dawson, 1973; Irvine & Dawson, 1978).

We have previously reported that lysosomes from rat liver can degrade pure phosphatidylinositol in a phospholipase C-like manner. The phospholipase C responsible is distinct from the other phospholipases of the lysosome, or other cell fractions, and is specific for phosphatidylinositol (Irvine *et al.*, 1977*a,b*, 1978). As there is considerable interest at present in the increased turnover of the phosphoinositol moiety

of phosphatidylinositol in physiologically stimulated tissues (for review see Michell, 1975) we have therefore considered a possible link between this phenomenon and increased lysosomal activity (Irvine *et al.*, 1978).

As our studies on phospholipid hydrolysis by lysosomal enzymes have been to date confined to pure phospholipids, we have now extended them to the hydrolysis of membranes by using as a model the hydrolysis of rat liver microsomal fraction. Apart from microsomal fraction being a useful source of isotopically labelled membrane, such a system is likely to be directly relevant to the digestion of membrane materials including endoplasmic reticulum in the autophagosome (Lloyd & Beck, 1974; Glaumann & Trump, 1975). We hope by these studies to establish whether or not the hydrolysis of phosphatidylinositol in a membrane is by a different mechanism from that of other glycerophospholipids. Secondly, as a preliminary step in investigating the hydrolysis of membranes by lysosomes, we hope to clarify the possible role of lysosomal phospholipases in releasing arachidonic acid for prostaglandin synthesis (Dawson & Irvine, 1978).

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Materials and Methods

Preparation of lysosomal enzymes

Large-granule soluble proteins were prepared by the method of Irvine *et al.* (1978). A more concentrated and purer lysosomal enzyme preparation was prepared by isolating lysosomes from rat liver by the method of Savant *et al.* (1964), by using about 200 g of liver from 16–20 male rats. The F IV fraction of Savant *et al.* (1964) was resuspended in 2–4 ml of water, frozen and thawed ten times, centrifuged for 1 h at 100 000g and the resulting supernatant was used as the lysosomal enzyme preparation for all but the preliminary experiments described in the present paper.

Preparation of ^{32}P -labelled microsomal fraction

Rats (one for each preparation) were injected intraperitoneally with 1 mCi of ^{32}P (carrier-free; The Radiochemical Centre, Amersham, Bucks., U.K.). After 18 h, liver microsomal fraction was prepared by the method of Higgins & Dawson (1977), except that the final pellet was suspended in 0.32 M-sucrose.

Preparation of myo-[^3H]inositol-labelled microsomal fraction

The preparation of microsomal fraction was as for ^{32}P -labelled microsomal fraction, but from rats injected 14 h previously with 100 μCi of myo-[^3H]inositol (sp. radioactivity 5.0 Ci/mmol; The Radiochemical Centre).

Assay of phospholipid hydrolysis

Unless stated otherwise, the assay system consisted of, in a final volume of 0.2 ml: 60 mM-potassium acetate buffer, pH 4.5; 6 mM-sodium EDTA; 5–9 μg of phospholipid P of microsomal fraction; and 0.38 mg of protein of the lysosomal enzyme preparation. Control tubes with no lysosomal enzymes were always assayed for each incubation condition. At the end of the incubation at 37°C, the tubes were transferred to an ice bath and 10 ml of chloroform/methanol (2:1, v/v) and 2 ml of 0.1 M- CaCl_2 were added. After centrifugation, the upper aqueous phase was washed three times with an equal volume of chloroform. The lower organic phase was washed three times with an equal volume of theoretical upper phase (Folch *et al.*, 1957) containing 0.04% CaCl_2 . The combined lower phases were concentrated to a small volume for separation of phospholipids by t.l.c.

T.l.c. of phospholipids

Precoated plates (silica gel 60 F₂₅₄; Merck A.G., Darmstadt, Germany) were spotted with phospholipids, and one-dimensional chromatographic separation was achieved by using chloroform/methanol/acetic acid/water (65:50:1:4, by vol.; Irvine &

Dawson, 1978). Two-dimensional chromatography used this solvent for the second dimension, and for the first dimension, chloroform/methanol/saturated ammonia (110:50:13, by vol.). ^{32}P -labelled phospholipids were located by radioautography, scraped into scintillation vials containing 15 ml of 'Fiscint' (Fisons, Loughborough, Leics., U.K.) and counted for radioactivity by liquid-scintillation counting.

Analysis of inositol-containing water-soluble products

myo-[^3H]inositol-labelled microsomal fraction was incubated with the lysosomal preparation exactly as above for ^{32}P -labelled microsomal fraction, but to increase the radioactivity in the upper phase, all quantities (except concentrations) in the incubation were increased by a factor of ten. The incubation buffer also contained glycerophosphoinositol, phosphoinositol and 1:2-cyclic phosphoinositol (final concentration of each 0.5 mM) to serve as traps that would minimize further degradation of myo-[^3H]inositol-labelled phosphate esters. Pairs of tubes (with and without lysosomal enzyme preparation) were incubated for 2 h, and the upper aqueous phase was obtained as described under 'Assay of phospholipid hydrolysis'. A known volume of each upper phase was loaded on columns (15 cm $\times$ 1 cm) of Dowex 1 (X8; Sigma Chemical Co., London S.W.6, U.K.) in the formate form (Hübscher & Hawthorne, 1957). The column was washed with 240 ml of water, and then eluted successively with sodium formate and sodium tetraborate as described by Hübscher & Hawthorne (1957). Out of each 8 ml fraction, portions were taken for liquid-scintillation counting, and for total phosphorus determination by the method of Rouser *et al.* (1970). The identities of the peaks obtained were determined by previous calibrations of the column with ^{32}P -labelled glycerophosphoinositol, phosphoinositol and 1:2-cyclic phosphoinositol (Dawson & Clarke, 1972) and myo-[^3H]inositol.

Release of fatty acids

The assay mixture was as described under 'Assay of phospholipid hydrolysis', except that non-radioactive microsomal fraction (prepared identically to ^{32}P -labelled microsomal fraction) was the substrate. The lower organic phases from the Folch separation were taken to dryness under N_2 , redissolved in 3 ml of hexane, and 3 ml of 0.1 M- Na_2CO_3 was added. After mixing, the tubes were centrifuged and the lower aqueous phases were collected. The upper hexane phases were washed three more times with 3 ml of 0.1 M- Na_2CO_3 and the combined lower phases from each were acidified with 3 M-HCl and then extracted three times with 5 ml of hexane. The hexane (combined upper phases) was removed by evaporation under vacuum, and the free fatty acids redissolved in 3 ml of diethyl ether/methanol (9:1, v/v), methylated

by the method of Schlenke & Gellerman (1960), and injected in diethyl ether solution on glass columns packed with diethylene glycol succinate in a gas-liquid chromatograph (Pye-Unicam Series 104). Separation was achieved isothermally at 196°C by using Ar as a carrier, and peak quantification was by the method of Carroll (1961). Total esterified fatty acids of the microsomal fraction were measured by alkaline hydrolysis of a phospholipid extract and then analysis of the fatty acids was by separation into hexane, and gas-liquid chromatography as described for released fatty acids (see above).

Detection of neutral lipid products other than non-esterified fatty acids

Unlabelled microsomal fraction was used as a substrate but, as for [^3H]inositol-labelled microsomal fraction, ten times the normal quantities were used. The chloroform extracts were concentrated and portions separated by t.l.c. with diethyl ether/ n -hexane/chloroform/acetic acid (55:40:5:0.2, by vol.) as solvent (Lines, 1963). To increase the separation of diacylglycerol from cholesterol, and monoacylglycerol from polar lipids, the plates were run twice in the same solvent. Lipids were estimated semi-quantitatively by charring the plates with 50% H_2SO_4 and comparing with fatty acids run as standards. As diacylglycerol liberated from microsomal phosphatidylinositol would be expected to be predominantly 1-stearoyl-2-arachidonoylglycerol (White, 1973), we used a 50:50 (w/w) mixture of stearic acid and arachidonic acid as a standard.

Results

Reproducibility

All experiments were repeated at least once. Quantitative variations were observed between different enzyme or substrate preparations, so statistical analyses are not given. Wherever data for different phospholipids or fatty acids appear on one Figure, however, they are always derived from experiments on the same enzyme and substrate preparation and are therefore directly comparable.

Hydrolysis of ^{32}P -labelled microsomal phospholipids by lysosomal enzymes

Preliminary assays of the hydrolytic capacity of lysosomal enzymes against a membrane substrate were made by using a preparation of large-granule soluble proteins (Irvine *et al.*, 1978). However, although this preparation is capable of hydrolysing phospholipids presented as pure substrates (Irvine *et al.*, 1978) it did not produce any measurable hydrolysis of any phospholipids in the microsomal fraction except a slow hydrolysis of sphingomyelin. Attempts to disrupt possible permeability barriers with lysophosphatidylcholine or by sonication of the

Fig. 1. Rates of hydrolysis of ^{32}P -labelled microsomal phospholipids by lysosomal enzymes

For experimental details of incubation see the Materials and Methods section. The data are from one experiment, but similar data were obtained in other experiments. Symbols: $\blacktriangle$, phosphatidylcholine; $\blacksquare$, phosphatidylethanolamine; $\bullet$, phosphatidylinositol; $\circ$, sphingomyelin.

microsomal fraction did not produce any increase in phospholipid hydrolysis, suggesting that under these conditions the dilution of the lysosomal phospholipases (Barrett & Heath, 1977; Irvine *et al.*, 1978) was too great for them to hydrolyse membranes to a measurable degree.

Therefore the method of Savant *et al.* (1964) was used to give a more concentrated and purer lysosomal preparation, and when only $1\text{ }\mu\text{g}$ of phospholipid P per tube is used (this was the amount employed in the preliminary experiments described above), extensive hydrolysis of all phospholipids present occurred in 2 h. At the same time the endogenous microsomal phospholipases appeared to be inactive under these incubation conditions.

To detect specific hydrolysis of any phospholipids during the initial stages of degradation, the amount of microsomal membranes was increased 5-fold and kept around this quantity for all subsequent experiments (see the Materials and Methods section). A time course of the hydrolysis under these conditions is plotted in Fig. 1. The results of such an experiment show that on a percentage basis sphingomyelin was most actively degraded, followed by phosphatidylethanolamine and phosphatidylcholine. The exact relative rates of hydrolysis of these latter two phospholipids varied between experiments, but phosphatidylethanolamine was always hydrolysed faster. Phosphatidylinositol was degraded to a lower extent and at a slower rate than the other phospholipids.

The addition of oleic acid (1 or $10\text{ }\mu\text{mol}/\mu\text{mol}$ of phospholipid), lysophosphatidylinositol ($1\text{ }\mu\text{mol}/$

4 μ mol of phospholipids), Tween 20 (0.125%, w/v) or Triton X-100 (0.2%, w/v) had no marked effect on any of these rates of hydrolysis. Palmitoyl-CoA (10 μ mol/ μ mol of phospholipid) had no effect on the hydrolysis of glycerophospholipids, but completely inhibited the hydrolysis of sphingomyelin (results not shown).

Fig. 2 shows the effect of Ca^{2+} on the hydrolysis of the phospholipids. It is apparent that although the

Fig. 2. Effect of Ca^{2+} on ^{32}P -labelled microsomal phospholipid hydrolysis by lysosomal enzymes

Experimental conditions and symbols are identical with those of Fig. 1; the incubation time was 2 h. No EDTA was added to the tubes containing Ca^{2+} , but those tubes plotted as containing zero Ca^{2+} contained EDTA (6 mM).

Fig. 3. Effects of pH on ^{32}P -labelled microsomal phospholipid hydrolysis by lysosomal enzymes

Experimental conditions and symbols are identical with those of Fig. 2. The buffers employed were acetate (pH 3.5–5.5), $\beta\beta'$ -dimethylglutarate (pH 6.0–7.2) and Tris/HCl (pH 7.2–7.8).

hydrolysis was inhibited, very high concentrations are required, higher than those found physiologically, and also higher than those needed to inhibit the hydrolysis of pure phospholipid substrates (Mellors & Tapell, 1967; Waite *et al.*, 1976; Irvine *et al.*, 1977b, 1978).

Fig. 3 shows a pH curve for the hydrolysis activity of the lysosomal enzymes. Sphingomyelin is degraded at a maximum pH value of 5–5.5, whereas glycerophospholipids are degraded more rapidly at a lower pH of around 4.5. The pH optima for phospholipid hydrolysis observed in the present paper are similar to those seen with pure substrates (Heller & Shapiro, 1966; Mellors & Tapell, 1967; Fowler & de Duve, 1969; Waite *et al.*, 1976; Irvine *et al.*, 1978).

The hydrolytic capacity of the lysosomal preparation was unaffected by heating to 60°C for 10 min, but was completely destroyed by keeping for 10 min at 100°C, a stability very similar to that recorded by Mellors & Tapell (1967).

Products of hydrolysis

Lysophospholipids. The degradation of phosphatidylcholine and phosphatidylethanolamine results in a considerable accumulation of their corresponding

Fig. 4. Production of lysophospholipids during hydrolysis of ^{32}P -labelled microsomal phospholipids by lysosomal enzymes

For experimental details see the Materials and Methods section. Similar data were obtained in an identical experiment. Symbols are identical with those in Fig. 1, except: Δ , lysophosphatidylcholine; $\square$, lysophosphatidylethanolamine.

lyso-compounds; after 2 h of incubation 60–70% remain in this form (Fig. 4).

The one-dimensional t.l.c. system used did not allow detection of any lysophosphatidylinositol produced from phosphatidylinositol, as the R_F of the former compound (Irvine *et al.*, 1978) was very similar to phosphatidylcholine. However, even after a two-dimensional separation, prolonged radioautographic exposure of the plate revealed the appearance of no unaccounted-for radioactive spot after the 2 h incubation, despite 60% hydrolysis of phosphatidylinositol, suggesting little accumulation of its lyso-derivative. Further support for this supposition came from independent experiments with *myo*-[2- 3 H]inositol-labelled microsomal fraction. The resulting t.l.c. separation of the eluted phospholipids was scraped off in 0.5 cm strips and counted for radioactivity by scintillation counting. Less than 4% of the phosphatidylinositol hydrolysed could be accounted for by a small peak of radioactivity in the lysophosphatidylinositol position.

Fatty acids. Fig. 5 shows the time course of the release of non-esterified fatty acids when non-radioactive microsomal fraction was hydrolysed by lysosomal enzymes. Despite quantitative variations, it is apparent that apart from some preferential release of the $C_{18:0}$ and $C_{22:6}$ fatty acid fractions, there appears to be little specificity in the hydrolysis.

Water-soluble *myo*-[2- 3 H]inositol esters. Analysis of the water-soluble radioactive compounds resulting

from the hydrolysis of *myo*-[2- 3 H]inositol-labelled microsomal fraction by lysosomal enzymes showed that quantitatively the most important products are inositol and phosphoinositol; there is also a small amount of glycerophosphoinositol (Fig. 6). If we assume that lysosomes contain no phospholipase D activity then the inositol must be derived from one of the two phosphate esters. Chemical analysis of the total phosphorus in the fractions containing glycerophospho[3 H]inositol showed that more than 96% of the original glycerophosphoinositol added to the incubation remained after incubation for 2 h with the lysosomal enzymes. A similar precise quantitative determination of phosphoinositol hydrolysis was not possible because it overlaps partially with the P_i peak, and there was an extensive production of P_i during the incubation; nevertheless, it was apparent that a substantial part of the added phosphoinositol trap had disappeared. To obtain further information on this point, non-radioactive microsomal fraction and [32 P]phosphoinositol, at the same concentration as described above, were incubated under identical conditions with the same lysosomal enzyme preparation. Here, when the P_i was separated from the phosphoinositol by ionophoresis (Dawson & Clarke, 1972) radioactive analysis showed that 50–60% of the [32 P]phosphoinositol had been hydrolysed to free inositol and P_i . As the free [3 H]inositol liberated from *myo*[2- 3 H]inositol-labelled microsomal fraction (Fig. 6) is approximately equal to the phospho[3 H]inositol liberated, it seems highly likely that nearly all the former is produced by hydrolysis of the latter. (It is relevant to add here that increasing the phosphoinositol trap in the incubation tubes to above 0.5 mM caused inhibition of the phosphatidylinositol degradation.)

Neutral lipids. Separation of the neutral-lipid products by t.l.c. (see the Materials and Methods section) showed that a small amount of diacylglycerol and monoacylglycerol accumulated after 2 h incubation with lysosomal enzymes. Neither of these were detected after incubation of microsomal fraction without lysosomal enzymes, and furthermore their production during incubation with lysosomal enzymes could not be attributed to hydrolysis of trace amounts of triacylglycerols in the microsomal fraction or lysosomes. In a typical experiment, if all the phosphatidylinositol hydrolysed had produced diacylglycerol (the percentage of this phospholipid was measured in a parallel experiment with 32 P-labelled microsomal fraction), the amount of diacylglycerol expected on the plate was approximately 18 μ g. About 0.7 μ g of diacylglycerol and 2 μ g of monoacylglycerol were actually detected. Clearly, as when using pure phosphatidylinositol as a substrate (Irvine *et al.*, 1978), most of any diacylglycerol formed was deacylated. However, the fact that it was detectable after a 2 h incubation with a highly concentrated

Fig. 5. Release of non-esterified fatty acids from the microsomal fraction by lysosomal enzymes

For methods of non-esterified fatty acid analysis and details of incubation see the Materials and Methods section. Release is expressed for each fatty acid as a percentage of the total esterified amount (see the Materials and Methods section). Similar results were obtained in an identical experiment. Symbols: ∇ , $C_{22:6}$ fatty acids; $\circ$, $C_{18:0}$ fatty acids; $\triangle$, $C_{20:4}$ fatty acids; $\bullet$, $C_{16:0}$ fatty acids; $\blacksquare$, $C_{18:2}$ fatty acids; $\square$, $C_{18:1}$ fatty acids.

Fig. 6. Water-soluble products of the hydrolysis of myo-[2-³H]inositol-labelled microsomal phospholipids by lysosomal enzymes

For details of the incubation and separation of the water-soluble products by ion-exchange resins, see the Materials and Methods section. Similar results were obtained in an identical experiment. Symbols: ○, microsomal fraction incubated without lysosomal enzymes; ●, microsomal fraction incubated with lysosomal enzymes. Fractions were of volume 8 ml. Abbreviations used: I, inositol; PI, phosphoinositol; GPI, glycerophosphoinositol.

lysosomal enzyme preparation (cf. Irvine *et al.*, 1978) shows that diacylglycerol released in a membrane is a less suitable substrate for the lipase than when it is activated by pure phosphatidylinositol (Karija & Kaplan, 1973).

Discussion

The main object of the present work lies in the study of the similarities and differences between the hydrolysis of a cellular membrane by lysosomal enzymes compared with our previous work and that of other workers that employed mainly isolated phospholipid substrates (for example, Mellors & Tapell, 1967; Fowler & de Duve, 1969; Waite *et al.*, 1976; Irvine *et al.*, 1978), whose physical state can have a great effect on enzyme activity and specificity (Dawson, 1973). In addition, the lysosomal enzyme preparation used often contained Triton WR-1339 (Waite *et al.*, 1976), which can inhibit lysosomal phospholipase activity (Irvine *et al.*, 1978). The major differences between using a membrane substrate and a pure phospholipid are probably due to the different physical environment caused by interaction of the phospholipid with other types of phospholipid, with membrane proteins and with cholesterol; it is doubtful if cellular phospholipases *in vivo* ever interact with a pure phospholipid or even a phospholipid mixture devoid of membrane protein. The choice of microsomal fraction as a membrane model had the advantage that a ready source of ³²P-labelled membrane was available, and that such a system was relevant to autophagosome digestion (Lloyd & Beck, 1974) and some pathological (Lloyd & Beck, 1974) and experimental (Glaumann & Trump, 1975) conditions in rat liver. Nevertheless, it is important to remember that the digestion of other types of cellular

membranes may be different both qualitatively and quantitatively from that of the endoplasmic reticulum.

There are several differences between the results recorded in the present paper and previous work using pure substrates. First, with a membrane substrate [Fig. 4 and the autodigestion of lysosomes (Waite *et al.*, 1976)] there is a marked accumulation of lysophospholipids, whereas Mellors & Tapell (1967) observed no such accumulation either with phosphatidylcholine or phosphatidylethanolamine as a substrate. The deacylation of phosphatidylinositol was also different in a membrane; with a pure substrate there is some accumulation of lysophosphatidylinositol, and the rate of total deacylation of phosphatidylinositol equals or exceeds that of the other glycerophospholipids (Irvine *et al.*, 1978). This may be, in part, a result of the ready dispersion of phosphatidylinositol as a pure phospholipid; in a membrane substrate the rate of phosphatidylinositol hydrolysis is less than that of phosphatidylcholine or phosphatidylethanolamine. The hydrolysis of pure phosphatidylinositol (Irvine *et al.*, 1978) is also more sensitive to Ca²⁺ than in membranes (Fig. 2), though the high binding capacity of pure phosphatidylinositol for Ca²⁺ (Hauser & Dawson, 1967) is probably the reason for the difference.

In the light of current interest in phosphatidylinositol turnover (Michell, 1975), perhaps the most interesting data concern the mechanism of hydrolysis of that phospholipid. The minimal accumulation of lysophosphatidylinositol and glycerophosphoinositol (Fig. 6) with the release of phosphoinositol and production of at least some diacylglycerol clearly show that, as with the pure substrate (Irvine *et al.*, 1977a,b, 1978), the predominant route of degradation of phosphatidylinositol is probably by phospholipase C-like hydrolysis.

It is therefore possible that in some physiological systems, where stimulated turnover of the phosphoinositol moiety of phosphatidylinositol (Michell, 1975) occurs in association with changes in fatty acyl-chain metabolism in this and other glycerophospholipids, these metabolic changes are merely due to increases in lysosomal activity. The known involvement of lysosomes in the response of the thyroid gland to thyrotropin, in the lectin-induced transformation of lymphocytes, and in phagocytosis by nucleocytes [see Dawson & Irvine (1978) for references], all three of which show changes in the turnover of the glycerol and fatty acyl moieties of phosphatidylinositol and other phospholipids (Scott *et al.*, 1968; Elsbach, 1968; Karnovsky & Wallach, 1971; Fisher & Mueller, 1971; Resch, 1976), makes these tissues good candidates for the stimulated phosphatidylinositol turnover being controlled by the lysosomal phosphodiesterase. In many other tissues that show stimulated phosphatidylinositol turnover, however, there are no such changes in the metabolism of other phospholipids (Michell, 1975), nor any evidence for an involvement of lysosomes; in these it would seem more likely that the cytoplasmic Ca^{2+} -dependent phosphatidylinositol phosphodiesterase (EC 3.1.4.10) is responsible.

We were previously unable to discount the possibility that the phosphatidylinositol phosphodiesterase of lysosomes and sphingomyelin phosphodiesterase (EC 3.1.4.12) were the same enzyme (Irvine *et al.*, 1978), though the identity of the enzyme responsible for phosphatidylinositol hydrolysis with an enzyme as highly specific as sphingomyelinase (Heller & Shapiro, 1966; Pentchev *et al.*, 1977) seems unlikely. The difference observed in the present paper in Ca^{2+} sensitivity (Fig. 2), palmitoyl-CoA inhibition (see above) and pH optimum (Fig. 3) all suggest a separation of the two activities, but conversely it could be argued that these differences are all due to specific effects on the substrate rather than the enzyme.

A final point on the different route of degradation of phosphatidylinositol by lysosomes compared with the other two major glycerophospholipids is that the predominant enzyme attacking phosphatidylinositol outside the lysosome is also a phospholipase C, the cytoplasmic Ca^{2+} -activated phosphatidylinositol-specific phosphodiesterase (EC 3.1.4.10) (Dawson, 1959; Kemp *et al.*, 1961; Thomson, 1967). Perhaps the extreme lytic potential of lysophosphatidylinositol makes deacylation of phosphatidylinositol undesirable and therefore, as with phosphatidic acid (Sturton *et al.*, 1978), the minor route of degradation. Glycerophosphoinositol does occur in living tissues, however (Seamark *et al.*, 1968; Koch & Diringer, 1974), and mammalian enzymes can produce it from phosphatidylinositol (Scott & Dawson, 1968; White *et al.*, 1971); thus as with phosphatidic acid, some

deacylation must occur, though the subcellular location of this is not known.

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