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THE NATURE OF THE PHOSPHOLIPASES A OF LUNG LAMELLAR BODIES

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SUMMARY

Phospholipases A_1 and A_2 from rabbit lung lysosomes have maximal activity at pH 4.0 (Fig. 1). Lamellar bodies from rabbit lung contain phospholipases A which resemble the lysosomal enzymes in being active at pH 4 and showing sensitivity to inhibition by calcium ions (80% inhibition at 10 mM Ca^{2+}). Lamellar body phospholipases hydrolyse dipalmitoyl phosphatidylcholine (containing 10% phosphatidylglycerol and thus approximating the phospholipid composition of mature lamellar bodies) at only 1% of their rate of hydrolysis of unsaturated molecules (Tables 1 and 2). This substrate preference corresponds to that shown by phospholipases from lysosomes.

SPECULATION

The substrate preferences of the lamellar body phospholipases may explain the ability of these enzymes to coexist with surfactant phospholipid in the mature organelle. In the developing lamellar body, phospholipase A_2 may have a role to perform in the removal of unsaturated phosphatidylcholine molecules and their remodelling to the dipalmitoyl species.

INTRODUCTION

Pulmonary surfactant, the material that stabilizes the air-liquid interface in mammalian lungs, is synthesized in the cuboidal cells of the alveolar epithelium. The surfactant is stored in these cells, prior to its secretion, in organelles known as lamellar bodies. Lung lamellar bodies have a well-established lysosomal nature (4; reviewed in 6). Since phospholipases A_1 (EC 3.1.1.32) and A_2 (EC 3.1.1.4) have been found in lysosomes from a variety of sources (reviewed in 12), it would be surprising if the enzyme complement of lamellar bodies did not include these phospholipases, and indeed we have shown previously that they both occur in lamellar bodies from rabbit lung (4).

The phospholipases A of lysosomes are soluble enzymes (i.e. released into the suspending medium when the membrane of the organelle is disrupted), have acid pH optima with little activity at alkaline pH, and are sensitive to inhibition by Ca^{2+} (12). In contrast, most non-lysosomal phospholipases A are dependent on Ca^{2+} for activity and are active only at alkaline pH. Several of them, for example the microsomal enzymes, are membrane bound.

Some confusion has arisen from the existence of these two kinds of phospholipase with such different optimal conditions. Garcia et al. (2) have reported that lamellar bodies do not contain phospholipases A, but since their incubations were carried out at pH 9 in the presence of 10 mM $CaCl_2$ it was unlikely that the lysosomal enzymes present in these organelles would be detected. Subsequent authors have failed to recognize this fundamental point, and have regarded the reports of Heath and Jacobson (4) and of Garcia et al. (2) as conflicting (7,9,10). Another investigation of phospholipase A activities in lamellar bodies used some incubations at pH 4, but in the presence of 10 mM $CaCl_2$, and the substrate for the assay was the phospholipid material of the lamellar body itself (7).

Our previous work (5) on phospholipases prepared from lysosomes showed that the predominant phospholipids from lung surfactant, dipalmitoyl phosphatidylcholine and phosphatidylglycerol, were not readily hydrolysed by the lysosomal enzymes, but did not investigate this property for phospholipases from lamellar bodies. The present report is chiefly concerned with phospholipases derived from lamellar bodies and demonstrates their marked preference for unsaturated rather than saturated phospholipids, and sensitivity to inhibition by calcium ions. The pH optima of phospholipases A_1 and A_2 from rabbit lung lysosomes are also presented.

MATERIALS AND METHODS

Preparation of extract containing lysosomal phospholipases A

The isolation of a lysosomal fraction from the lungs of New Zealand White rabbits, and the preparation from this of a soluble extract containing phospholipases A_1 and A_2 were as described by Heath and Jacobson (5).

Preparation of lamellar bodies

Rabbit lungs were homogenized in 0.33 M sucrose - 10 mM Tris.HCl (pH 7.5), and a lamellar body fraction was obtained by the technique of Engle et al. (1). The organelles were resuspended in 1 mM Na_2EDTA - 1 mM $NaHCO_3$ and disrupted by freezing and thawing, and dialysis overnight. If required, a soluble extract was prepared as previously described (4).

Phospholipase assays

Assays were performed as described previously (4,5). Substrates were prepared as liposomes in 80 mM sodium acetate buffer (pH 4.0) containing either 10 mM Na_2EDTA or 20 mM calcium acetate, and 0.1 ml portions were incubated with 0.1 ml of lamellar body preparation. For determinations of pH optima, liposomes were prepared in 60 mM sodium phosphate at pH 3.0, 4.0, 5.0, 6.0 and 7.0, and were incubated with the enzymes prepared from lysosomes.

RESULTS

Phospholipases A_1 and A_2 from rabbit lung lysosomes each have maximal activity at pH 4.0 (Fig. 1).[2] The peak is sharp for A_1, but A_2 shows 85% of maximal activity at pH 5.0. The activity of each enzyme is less than 15% of maximal at pH 6.0 and above.

The activities of phospholipases A of lamellar bodies are shown in Table 1, and of the soluble extract from lamellar bodies in Table 2. Both saturated and unsaturated phosphatidylcholines were used as substrates, in the presence and the absence of Ca^{2+}. Calcium ions (10 mM) were inhibitory, not more than 20% of the activity remaining. When the substrate consisted of equal parts of saturated and unsaturated molecules, almost two-thirds of the activity was directed against the latter (Table 2). Activity against dipalmitoyl phosphatidylcholine containing 10% phosphatidylglycerol (and thus resembling the phospholipid composition of the mature lamellar body) was very much less than against the unsaturated species (Tables 1 and 2). The specific activity of phospholipase A in the soluble extract was 0.5 nmol.min^{-1} per mg protein under optimal conditions.

DPPC/PG di[^{14}C]palmitoyl phosphatidylcholine : phosphatidylglycerol, 9:1 mol/mol

[2]Expressed as a percentage of the value for POPC as substrate in the absence of calcium ions

[3]Given where available

DISCUSSION

The pH optima of lysosomal phospholipases A from rabbit lung are very similar to those described for phospholipases from other lysosomal sources (12). The presence of these enzymes in subcellular fractions will be overlooked if incubations are made at pH 8 or 9. These phospholipases are also sensitive to inhibition by calcium ions (5,12), a common component of phospholipase assay media.

The enzymes of lamellar bodies can be demonstrated at pH 4.0 in the absence of Ca^{2+}. The presence of calcium ions (10 mM) causes marked inhibition (Tables 1 and 2). Substrates rich in dipalmitoyl phosphatidylcholine are hydrolysed much less readily than unsaturated species. A similar result was found in earlier studies of phospholipases prepared from lysosomes (5). Acidic phospholipids have also been shown to be poor substrates (12) and we showed previously that one of them, phosphatidylglycerol, appears to inhibit the hydrolysis of dipalmitoyl phosphatidylcholine by these enzymes (5). The phospholipid stored in mature lamellar bodies consists principally of dipalmitoyl phosphatidylcholine and phosphatidylglycerol, so it forms a particularly poor substrate for the detection of lysosomal phospholipases.

The de novo synthesis of phosphatidylcholine in the lung produces mostly molecules with an unsaturated fatty acyl residue at the 2-position, and not the dipalmitoyl species required for surfactant. These molecules must therefore be converted to dipalmitoyl phosphatidylcholine by remodelling processes (reviewed in 11). Remodelling consists of the removal of the unsaturated fatty acid by a phospholipase A_2, followed by its replacement with a palmitoyl residue by the action of an acyltransferase. As we have suggested previously (4-6) the phospholipases in lamellar bodies may be useful in the early stages of lamellar body formation, when unsaturated phosphatidylcholines must be removed, phospholipase A_2 facilitating the remodelling of these unsaturated molecules to dipalmitoyl phosphatidylcholine. In the mature organelle, however, the phospholipases should ideally be inactivated. The unfavorable physicochemical nature of these phospholipids may prevent the enzymes from acting (5), as may the relatively dehydrated state of the interior of the lamellar body (3,8).

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TABLE 1
Phospholipase A activities of disrupted, but unfractionated, rabbit lung lamellar bodies

Substrate[1]	Ca^{2+} (mM)	Phospholipase A activity[2]	Activity ratio[3] A_1:A_2
POPC	0	100	0.8:1
POPC	10	3	0:1
DPPC/PG	0	1	
DPPC/PG	10	0	

TABLE 2
Phospholipase A activities of the soluble extract from rabbit lung lamellar bodies

Substrate[1]	Ca^{2+} (mM)	Phospholipase A activity[2]	Activity ratio[3] A_1:A_2
POPC	0	100	2:1
POPC	10	20	0:1
USPC/DPPC	0	60	
DPPC/USPC	0	34	
DPPC/PG	0	1	
DPPC/PG	10	0	

[1]Abbreviations used:
POPC 1-palmitoyl-2-[^{14}C]oleoyl phosphatidylcholine dispersed in egg phosphatidylcholine
USPC/DPPC unsaturated [^{14}C]phosphatidylcholine : dipalmitoyl phosphatidylcholine, 1:1 mol/mol
DPPC/USPC di[^{14}C]palmitoyl phosphatidylcholine : unsaturated phosphatidylcholine, 1:1 mol/mol

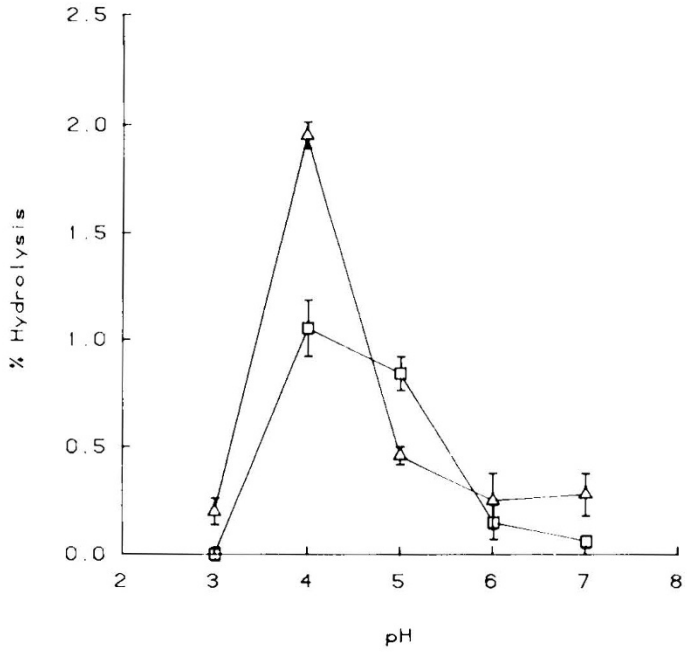

Fig. 1. Effect of pH on the activity of phospholipases A from rabbit lung lysosomes. Assays of phospholipases A_1 (△——△) and A_2 (□----□) were performed as described in the text. Vertical lines represent SEM (n=4).

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