PHOSPHOLIPID COMPOSITION OF SOME AMINE STORAGE GRANULES

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1. Introduction

Several mammalian tissues or cells contain subcellular organelles which accumulate and store biogenic amines, e.g., adrenal medulla chromaffin granules, platelet dense granules, mast cell granules, and anterior pituitary granules. The similarity among these granules is not restricted to a high content of biogenic amines, but extends to the presence of ATP, peptide hormones, and large M_r acidic molecules implicated in the storage complex as well; also to the extremely low membrane permeability to ions, to the maintenance of a distinctly acidic intragranular pH of 5.5–6.1, and to the release of amines by a calcium-dependent stimulus coupling mechanism [1–6]. Adrenal chromaffin granules are known to be characterized by an unusually high amount (up to 17 mol%) of lysophosphatidylcholine in their membranes [7–13], and this observation has led to much speculation as to the role of lysophospholipid in secretory granule membranes. The measurements have also generated controversy over whether the high lysophosphatidylcholine content is a manifestation of post-mortem artifacts. In view of this controversy, recently fueled by several reports [14,15], we have carried out a comparative study of the phospholipid composition of several amine storage organelles. This comparison, which utilized similar isolation procedures and one standardized analytical technique for phospholipid measurement, included bovine chromaffin granules purified through different density gradients, chromaffin granules isolated from a human pheochromocytoma (an adrenal medullary tumor), serotonin dense granules from pig platelets, bovine anterior pituitary granules, and intact rat peritoneal mast cells.

The results indicate that the phospholipid composition of bovine chromaffin granules is independent of the gradient purification technique, and that the lysophosphatidylcholine content of the bovine and pheochromocytoma chromaffin granules and intact rat mast cells is very high. By contrast, however, platelet and pituitary granules do not contain high lysophosphatidylcholine levels. These results are discussed with respect to previously published values from different laboratories, and some possible physiological implications are presented.

2. Materials and methods

Chromaffin granules from bovine adrenal glands were isolated in 0.27 M sucrose, 10 mM Tris–maleate (pH 7.0). Purification was carried out by centrifugation through either 1.6 M sucrose [16], Ficoll-sucrose-^2H_2O [17], or Percoll–sucrose [18] gradients.

Pheochromocytoma tissue was removed at the time of surgery and immediately used to prepare chromaffin granules. These were purified through a Percoll–sucrose gradient [18].

Mast cells were isolated from the peritoneal cavity of rats as in [4].

Serotonin-containing granules were isolated from pig platelets as in [1].

Secretory granules from bovine pituitary glands were obtained through a modification (R. G. J., unpublished observations) of the Percoll method [18]. Preparations obtained by this procedure (which employs a Percoll concentration 2% higher than that used in the purification of chromaffin granules) appeared extremely pure as judged by electron microscopy (not shown).

The above materials were isolated in Philadelphia and shipped in dry ice to Utrecht, where they arrived the next day still completely frozen. Lipids were

Table 1
Phospholipid composition (%)[a] of chromaffin granules

Source	Isolation	L-PC	PC	PE	PI	PS	SPH	Others
Bovine adrenal medulla[b]	Sucrose	14.4 ± 0.5	24.8 ± 1.1	34.5 ± 1.8	2.9 ± 0.2	7.9 ± 1.6	15.6 ± 2.0	–
	Ficoll	15.7 ± 0.5	24.7 ± 0.8	33.7 ± 1.1	2.9 ± 0.1	7.9 ± 0.2	15.2 ± 1.4	–
	Percoll	16.0 ± 1.2	25.4 ± 0.1	33.2 ± 1.0	2.8 ± 0.1	7.9 ± 0.6	14.7 ± 2.0	–
Human pheochromocytoma[c]	Percoll	23.1	16.1	29.0	1.1	8.5	16.0	5.0[d]

[a] Values are expressed as percentage of total lipid phosphorus recovered: L, lyso; P, phosphatidyl; C, choline; E, ethanolamine; I, inositol; S, serine; SPH, sphingomyelin
[b] Mean ± SD of 4 determinations in one preparation
[c] Mean of 2 determinations in one preparation
[d] Human pheochromocytoma granules contained 4.8% lysophosphatidylethanolamine and 0.2% of cardiolipin + phosphatidic acid

extracted according to [19] and phospholipid classes were resolved by two-dimensional thin-layer chromatography [20]. Phosphorus in silica scrapings was determined as in [21].

3. Results

Table 1 summarizes the phospholipid composition of bovine chromaffin granules obtained from the same adrenal medulla homogenate but purified through different density gradients. It is obvious that the isolation procedure had no significant effect on the measured phospholipid composition of the final granule preparation; a large and equivalent (14.4–16.0 mol%) amount of lysophosphatidylcholine was measured under the various conditions.

The phospholipid composition of chromaffin granules isolated from a human pheochromocytoma were analyzed because it was possible to isolate the granules extremely rapidly after removal from the body, minimizing any tendency for post-mortem decay (table 1). While by definition a tumor, the pheochromocytoma can be highly differentiated and resemble normal chromaffin tissue. In fact, granules isolated from this pheochromocytoma contained higher catecholamine levels, maintained a lower ion permeability, and exhibited better energy-linked functions than the

Table 2
Phospholipid composition (%)[a] of peritoneal mast cells, platelet granules and pituitary granules

Material	L-PC	PC	PE	PI	PS	SPH	Others
Rat peritoneal mast cells[b]	6.6 ± 1.3	35.1 ± 3.1	19.2 ± 2.2	4.0 ± 0.6	9.5 ± 0.9	18.3 ± 2.2	6.0[c]
Serotonin granules from pig platelets[d]	0.5 ± 0.8	36.5 ± 4.7	22.8 ± 3.3	4.3 ± 1.2	10.3 ± 1.8	24.9 ± 2.4	–
Bovine pituitary granules[e]	–	41.2	26.2	3.2	6.3	21.7	1.5[f]

[a] Values are expressed as percentage of total lipid phosphorus recovered: Abbreviations as in table 1
[b] Mean ± SD of one determination in 3 preparations
[c] Rat peritoneal mast cells contained 3.1 ± 1.8% lysophosphatidylethanolamine and 2.9 ± 2.8% cardiolipin
[d] Mean ± SD of 5 determinations in 4 preparations
[e] Mean of 2 determinations in one preparation
[f] Bovine pituitary granules contained 1.5% cardiolipin

bovine chromaffin granules [22]. The lysophosphatidylcholine (lyso-PC) content of these membranes constituted almost 25% of the total phospholipids.

There appears to be good agreement between the data in table 1 and that reported by several research groups, each employing different methods for granule purification and/or phospholipid separation and analysis [7–13,21]. In addition, the results are consistent with the notion that the presence of lyso-PC within the chromaffin granule is not due to post-mortem autolysis.

Applying similar preparation techniques as were utilized for the isolation of the chromaffin granules, several other amine containing sub-cellular organelles were prepared, including porcine platelet dense granules, bovine anterior pituitary granules, and intact rat peritoneal mast cells. Analysis of the phospholipid composition in these preparations (table 2) indicate that essentially no lysophospholipids exist in the serotonin or anterior pituitary granules. On the other hand, within the intact mast cells the lyso-PC content was measured to be 6 mol%. Existing methodological limitations for isolation of intact mast cell granules in significant quantities precluded direct verification of the lyso-PC levels of the storage granule membrane. However, assuming that the lyso-PC is predominantly present within the granular membrane and that ~1/3rd of the total mast cell membrane is associated with the histamine-storing organelle (R. G. J., unpublished), the latter membrane may have high lyso-PC levels comparable to those of chromaffin granules.

4. Discussion

The finding that bovine chromaffin granule membranes possess the highest content of lysolipid contained in any mammalian membrane has led to a search for the enzymatic regulation of this level and for its physiological significance; several theories relating to secretory phenomena are founded upon the lysolecithin content within the membrane [7,24–27]. Evidence is rapidly accumulating that amine containing subcellular organelles maintain similar properties with regard to structure, function, bioenergetics, and secretion. With these thoughts in mind, we attempted to address two questions:

(1) Does the chromaffin granule in fact posses a high content of lyso-PC, in vivo;

(2) Is this lyso-PC content a universal property of amine containing subcellular organelles?

The approach was to prepare the granules under similar conditions of isolation media, temperature, time of isolation, and preparative density gradients in a laboratory which routinely isolates these subcellular fractions, and to analyze all samples in a laboratory where expert lipid analysis is possible.

The results from table 1 indicate that the lyso-PC measured is independent of the isolation procedure and is probably not due to post-mortem decay, this is supported by the high lyso-PC content in chromaffin granules rapidly isolated from a human pheochromocytoma. These conclusions conflict with 2 recent reports which concluded that the measured lyso-PC was secondary to post-mortem autolysis:

(1) In [14] only 4.4% was reported lyso-PC in bovine chromaffin granules. However, in addition to the variance of the lyso-PC data in [14] with literature values, much higher levels for PE (51.7% *vs* 33%) and a lower sphingomyelin content (8.9% *vs* 14%) were obtained than other investigators. In [14] only trace amounts of lyso-PC in granules isolated from whole adrenal glands of the guinea pig were reported and, on the basis of this result, lyso-PC accumulation in bovine chromaffin granules was proposed to be due to post-mortem autolysis. An alternative explanation for the low lyso-PC content of these guinea pig chromaffin granules could lie in the purity of the preparation, which contained the characteristic mitochondrial phospholipid cardiolipin in amounts comparable to the mitochondrial fraction itself [14]. Other investigators have considered this possibility and have approached the question in a similar fashion, namely by isolating granules from the adrenals of laboratory animals in order to minimize post-mortem autolysis. As a result of this approach the lyso-PC contents of rat [8] and rabbit [13] chromaffin granules have been reported to amount to 15.4% and 10.4%, respectively.

(2) In [15], the lyso-PC content of bovine adrenal medullae, bovine chromaffin granules, and intact rabbit adrenal glands was determined and it was concluded by implication that granular lyso-PC is produced in adrenals not during isolation of the granules from the homogenate, but during the time between killing of the animal and processing of the glands at 4°C. Unfortunately, these conclusions were based on low levels of lyso-PC in lipid extracts of total glands rather than on analysis of the granules themselves, and do not provide an explanation for the results of

previous analysis of laboratory rat [8] and rabbit [13] chromaffin granules, which indicated high levels of lyso-PC comparable to those of bovine chromaffin granules. Here, this controversy was circumvented by the use of human pheochromocytoma tissue, which was excised, placed on ice, and processed with near-maximal rapidity.

In relation to the putative possibility of post-mortem decay, several other findings deserve consideration:

(i) Isolation of the granules under optimal conditions (i.e., pheochromocytoma) produced high lyso-PC contents (table 1 and [23]);
(ii) Variations in the gradient material utilized which affected the osmolality, ionic strength, and duration of isolation were without effect (table 1).
(iii) General unrestricted autolysis of phospholipids (as observed in the pancreas; see [28–30]) would lead to lyso-derivatives of all phospholipid classes of different subcellular membranes; by contast, it has been firmly established that chromaffin granules are the only subcellular structures isolated from adrenal medulla containing appreciable levels of lysolipid and, moreover, the lysolipid is almost exclusively lyso-PC [7,8,15,31,32];
(iv) The topographic localization of lyso-PC in the inner monolayer of the chromaffin granule membrane [12,33], together with its apparently restricted transbilayer movement [12], does not lend credence to the hypothesis of post-mortem lyso-PC production of extragranular phospholipases.

However, studies on the subcellular distribution of phospholipase activities in adrenal medulla have so far not provided indications for the presence of intragranular phospholipase A [34,35]. The fact that chromaffin granules from healthy adrenals [8] and from pheochromocytoma [23] contain only trace amounts of free fatty acids is also not in line with the production of lyso-PC by a hypothetical intragranular phospholipase A. In the meantime it remains an interesting and as yet unresolved question by what mechanism and at what stage of development lyso-PC is formed in the chromaffin granule membrane.

The lyso-PC levels found in intact mast cells (table 2) are significantly higher than those in [36]. Assuming that lyso-PC is predominantly present in the granules and not in the plasma membrane, as in section 3, mast cell granule membranes appear to have lyso-PC levels nearly as high as those of chromaffin granules. Serotonin granules from pig platelets are virtually devoid of lyso-PC (table 2). This agrees with the reported phospholipid composition of rabbit platelet 5-hydroxytryptamine granules [9]. Secretory granules isolated from bovine adenohypophysis also do not show the presence of pre-existing lysophospholipids (table 2). Although 5% of the total lipid phosphorus as lyso-PC in prolactin secretory granules was reported [37] significant differences between this value and the corresponding data for the microsomal fraction was observed. The phospholipid composition of granules obtained from the pars nervosa showed that lyso-PC did not account for >1% of total lipid phosphorus [38].

Other examples of exocytotic systems which do not reveal high lysophospholipid contents can be found in the literature. Thus, synaptic vesicles from adult rat brain [39], guinea pig cerebral cortex [40], and *Torpedo marmorata* electric organ [40] were found to contain ⩽1.5% lyso-PC. Taken together the available data indicate that high levels of preformed lyso-PC are not a universal characteristic of biogenic amine secretory systems.

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