The Composition of Phospholipid and Phospholipase-A Activity in the Rat Submaxillary Gland (Part 1)

By

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Introduction

It is understood that phospholipids spread widely in the mammalian tissues, and major proportion of phospholipids in cells is present in the particulate fractions where they are considered to be essential structural components of cell and intracellular membrane. It has been recognized that three main phospholipids are phosphatidylcholine, phosphatidylethanolamine (phosphatidylserine) and sphingomyeline.

It is well known that phospholipase A can act on phosphatidylcholine with release of one mole of fatty acid and one mole of lysophosphatidylcholine, and spreads widely in the mammalian tissues. For example, there is a high degree of its activity in the pancreas and small intestine, some degree of it in the testis, spleen, lung and liver and a small degree of it in the brain, heart muscle and skeletal muscle [1, 2].

Recently, in the study of membrane permeability in the living cell, phospholipases were used as a prominent means for the study in its field [3, 4, 5]. In our previous report, MURAKAMI has reported that phospholipase A may have a role in the pancreatic juice secretion process [6]. Moreover, HIGUCHI et al. have reported that anionic amphipathic substances (dodecylsulfate, hexadecylsulfate and taurocholate) increase the pancreatic juice secretion [7]. OSADA et al. have reported that those substances increase not only bile secretion but also the parotid salivary secretion [8]. However, phospholipase A activity in the salivary gland is not clear, and moreover, relatively little is known about the physiological role of the enzyme. In the present experiment, our first aim is to examine the composition of phospholipids in the rat submaxillary gland and, secondly, to confirm phospholipase A activity in the rat submaxillary gland.

Materials and Methods

1. Animals

Male 6-week-old rats of Donryu strain (body weight, around 100g) were supplied by Nippon Rat Co.

2. Materials

Pure phosphatidylcholine was crude phosphatidylcholine purchased from Merk

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and purified in our laboratory. Silica gel H was also obtained from Merk. All reagents used in these experiments were guaranteed reagents. Rat submaxillary gland tissues were carefully removed from the animals, and immediately after being sacrificed by cutting the carotid artery, were cooled with chipped ice. The lipids were extracted by homogenizing the tissues in a waring blender containing chloroform-methanol mixture (2:1, V/V) according to method of Folch et al. [9]. Phospholipase A was extracted according to the methods of Magee et al. and Jennifer et al. as shown in Figs. 1, 2, 3 and 4 [2, 10].

Fig. 1. Extraction of phospholipase A in the rat submaxillary gland

Fig. 2.

Fig. 3.

Fig. 4.

3. Methods of Assay

The extracted total tissue lipids were dissolved in 0.1ml anhydrous chloroform per 0.1g of tissue (wet weight), and 10 μ l of the solution was then applied to silica gel layered glass. Separation of phospholipids was carried out by T.L.C. Chromatogram was developed in chloroform-methanol-water (65:25:4, V/V) for 20 min. in a room temperature. The determination of phosphorus was carried out according to the method of Chan et al. [11]. Spectrophotometer used for determination of phosphorus was spectrophotometer HITACHI Model 124, (Hitachi Seisakusho Co. Ltd.).

4. Estimation of Phospholipase A

Fraction A and fraction D were used in this experiment as the source of enzyme. Phospholipase A activity was measured by estimating the decrease in concentration of phosphatidylcholine used as the substrate, and 2mg of phosphatidylcholine was used.

5. Estimation of Phosphatidylcholine

4ml of chloroform-methanol (2:1, V/V) was added to 1ml of reaction mixture, after shaking for 5 min. in a room temperature, and removing the upper layer, the lower layer was transferred to another flask, evaporated by the rotary evaporator to dryness, and dissolved in 0.2ml anhydrous chloroform and then 10 μ l of the solution was used in T.L.C., and chromatogram was developed in chloroform-methanol-water (65:25:4, V/V) for about 20 min.

Results

It was established that PC, PE, Sph and Lyso-PC were present in the rat submaxillary gland tissues, as shown in Tab. 1 and Figs. 5 and 6. The composition of these phospholipids was as follows: PC was 44.4% of total phospholipids, PE 22.6%, Sph 6.0% and Lyso-PC 3.5%. In the submaxillary gland tissue, major constituents of phospholipids were PC, PE and Sph. Trace of Lyso-PC was present. Using fraction A shown in Fig. 1 as the source of enzyme, it was recognized that phospholipase A activity was present, and hydrolysis-rate of control-PC was 42.5% and 48.4% as shown in Tab. 2. It was confirmed that phospholipase A activity was present in the fraction D from the submaxillary gland, as shown in Tab. 3 and Phot. 1, and its hydrolysis-rate of control-PC was 34.9%, 34.6% and 37.6%.

Table 1. Phospholipids in the rat submaxillary gland tissue.

	Tp	Unknown	PE	PC	Sph	Lyso-PC
P mg/lg wet tissue mean $\pm$ SE	1.22 $\pm$ 0.04	0.04 $\pm$ 0.01	0.28 $\pm$ 0.03	0.54 $\pm$ 0.05	0.07 $\pm$ 0.01	0.04 $\pm$ 0.01
% of Tp		3.5	22.6	44.4	6.0	3.5

Tp: Total phospholipids

Fig. 5. Chromatogram of total lipids in the rat submaxillary gland tissue.

Fig. 5. Chromatogram of total lipids in the rat submaxillary gland tissue.

Fig. 6. Composition of phospholipids in the rat submaxillary gland tissue.

Table 2. Hydrolysis of phosphatidylcholine by the fraction A as a source of enzyme

Exptl. No.	1	2
P mg of control PC	0.188 $\pm$ 0.004*	0.186 $\pm$ 0.004*
P mg of PC+enzyme	0.108 $\pm$ 0.007*	0.096 $\pm$ 0.003*
Hydrolysis-rate %	42.5	48.4

* mean $\pm$ SE

Mean value was obtained in 5 experiments.

Reaction system contained 2 mg of PC and 5 ml of fraction A in 0.2 ml of 0.1 M Tris-HCl buffer (pH 7.4), ether 2 ml, 0.04 M CaCl₂.

Incubation temp.: Room temp. (Ca. 25°C).

Incubation time: 5 min.

Table 3. Hydrolysis of phosphatidylcholine by the fraction D
as a source of enzyme

Exptl. No.	1	2	3
P mg of control PC	$0.188 \pm 0.007^*$	$0.188 \pm 0.166^*$	$0.202 \pm 0.008^*$
P mg of PC+enzyme	$0.122 \pm 0.008^*$	$0.123 \pm 0.005^*$	$0.126 \pm 0.007^*$
Hydrolysis-rate %	35.1	34.9	37.6

* mean $\pm$ SE

Mean value was obtained in 5 experiments.

Reaction system contained 2 mg of PC and 2 mg of fraction D
in 0.2 ml of 0.1 M Tris-HCl buffer (pH 7.4), ether 2ml, 0.04 M CaCl_2 .

Incubation temp.: Room temp. (Ca. 25°C).

Incubation time: 5 min.

Photo. 1. Hydrolysis of phosphatidylcholine by the fraction D.

Reaction system contained 2 mg of PC and 2 mg of fraction D in 0.2 ml of 0.1 M Tris-HCl buffer pH 7.4 ether 2 ml, 0.04 M CaCl_2 . Incubation temp.: Room temp. (Ca. 25°C), Incubation time: 5 min. Developing solvent is a mixture of chloroform-methanol-water, 65:25:4, (V/V).

Discussion

We demonstrated the composition of phospholipids in the rat submaxillary gland were phosphatidylcholine, phosphatidylethanolamine (possibly containing phosphatidylserine) and sphingomyeline. Trace of lysophosphatidylcholine and unknown phospholipid were present. These results show that this was similar to the composition of phospholipids in various tissues of animals, as mentioned earlier by DAWSON [12].

Furthermore, we attempted to estimate the phospholipase A activity of these unpurified (fraction A) and purified (fraction D) tissue extracts by measuring the decline in the concentration of added substrate (purified phosphatidylcholine), and the fraction A or D liberated free fatty acid from the added substrate and lysophosphatidylcholine was formed by action of the fraction A or D, and the concentration of added substrate was decreased. From the above results, it was recognized that phospholipase A activity was present in the rat submaxillary gland. But fraction A was similar to fraction D in the hydrolysis-rate of the added substrate. These results suggest that the fraction A may contain other phospholipases (e.g. phospholipase B, C and D).

The role of phospholipases contained in cell membrane has been discussed in the field of membrane permeability mechanism of substances [13] and in the relation between the changes of phospholipids composition and of phospholipase activities in the nerve fiber during excitation [14, 15]. The fact that phospholipase A activity was also present in submaxillary gland seems to be of interest in clarifying salivary secretion mechanisms.

Summary

1. A detailed composition of phospholipid has been examined in the rat submaxillary gland. It was recognized that there is phosphatidylcholine, phosphatidylethanolamine (possibly containing phosphatidylserine) and sphingomyeline as a major constituent of phospholipids, that there is trace of lysophosphatidylcholine and unknown phospholipid.

2. The phospholipase A activity of unpurified tissue extract (fraction A) or purified tissue extract (fraction D), as the source of enzyme has been examined. The fraction A or fraction D hydrolysed phosphatidylcholine as a substrate, and liberate free fatty acid and formed lysophosphatidylcholine. From above results, it was established that phospholipase A activity is present in the rat submaxillary gland.

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