Hemagglutinating activity of phosphatidylserine

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1. INTRODUCTION

In [1] we reported the structure and hemagglutinating activity of an ornithine-containing lipid of *Bordetella pertussis*. The proposed structure of the aminolipid is 3-hydroxyhexadecanoic acid amide-linked to ornithine and esterified to the second hexadecanoic acid. The aminolipid strongly agglutinated human and rabbit erythrocytes.

Here, I describe the hemagglutinating activity of phosphatidylserine which is known as one of the membrane phospholipids. The activity was found because the structure of phosphatidylserine was similar to that of the ornithine-containing lipid of *B. pertussis*.

2. MATERIALS AND METHODS

L-α-Phosphatidyl-L-serine from bovine brain (purity 99%) was purchased from Sigma (St Louis MO). Purity was confirmed by silica gel thin-layer chromatography with a solvent system chloroform–methanol–water (65:25:4, by vol.).

Hemagglutinating activity of various lipids was determined by the 2-fold dilution method in microplates with fresh human and various animal erythrocytes at 0.5% (v/v), 28°C for 2 h in 0.01 M phosphate buffer–0.85% NaCl (pH ~6.0). The test for inhibition of hemagglutination by phosphatidylserine was carried out in microplates with fresh chicken and type O human erythrocytes at 0.5% (v/v), with lipids, amino acids and sugars used as inhibitors, in 0.01 M phosphate buffer–0.85% NaCl (pH ~6.0). After the inhibitory substances had been mixed with phosphatidylserine and incubated at 37°C for 1 h, the reaction mixtures were mixed with erythrocytes and further incubated at 28°C for 2 h. Lipids used in the above test, except phosphatidylserine, were pre-sonicated in 0.01 M phosphate buffer–0.85% NaCl (pH ~6.0) for 1–2 min. Phosphatidylserine was sonicated for 20–30 s, because it is unstable to sonication. Lipids, amino acids and sugars were of the highest purity available commercially.

Fig.1. Photograph of agglutination of chicken erythrocytes by phosphatidylserine; × 300.

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3. RESULTS

Phosphatidylserine definitely exhibited hemagglutinating activity and strongly agglutinated human, rabbit, green monkey, chicken and BALB/c mouse erythrocytes (table 1 and fig.1).

Table 1

Minimum hemagglutinating concentration of phosphatidylserine

Erythrocytes	Lipid (μg/ml)
Human	
Type O	1
Type A	1–2
Type B	1–2
Rabbit	0.5
Green monkey	2
Sheep	125
Horse	125
Guinea-pig	125
Mouse (BALB/c)	2
Chicken	4

The procedure is described in section 2

Table 2

Hemagglutination inhibition test with lipids, amino acids and sugars

Inhibitory substance	Minimum conc. (mg/ml) completely inhibiting hemagglutination by 6 μg phosphatidylserine/ml
Phosphatidyl-choline	0.02
Asp	0.03
Glu	0.03
Sialic acid	0.03
His	0.08
Sphingomyelin	0.08
Triolein	0.9
Methyl oleate	0.9
Cholesterol	0.9
Arg	2.0
Lys	2.0

Substances non-inhibitory at < 4 mg/ml: Ala, Thr, Phe, Tyr, Leu, Trp, Orn, Cys, CySH, Gln, Met, Pro, L-Fucose, D-Gal, D-Glc, D-Man, Lactose, D-GlcNAc, D-GalNAc, tripalmitin, phosphatidylethanolamine, palmitic acid, stearic acid

The hemagglutination was strongly inhibited by phosphatidylcholine, sialic acid, aspartic acid, glutamic acid, sphingomyelin and histidine (table 2). Difference was not found between chicken and type O human erythrocyte.

No breakdown of phosphatidylserine by sonication was observed (fig.2). The R_f-value of sonicated phosphatidylserine was the same as that of authentic phosphatidylserine and no fatty acids or lysophosphatidylserine were produced. The spots of the sonicated phosphatidylserine were ninhydrin-positive.

Phosphatidylethanolamine, cardiolipin, phos-

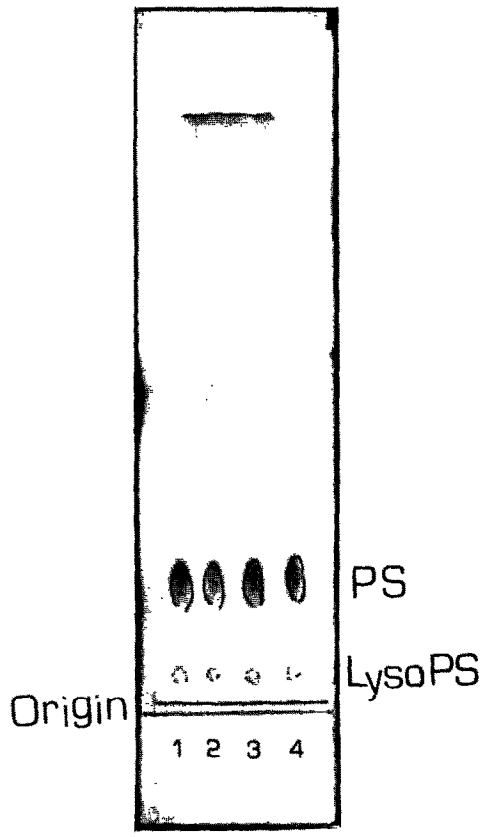

Fig.2. Thin-layer chromatogram of sonicated phosphatidylserine. After phosphatidylserine had been sonicated for 20 s (sample no.2), 1 min (no.3) or 4 min (no.4) and extracted with chloroform–methanol (2:1, v/v), the products were analyzed by thin-layer chromatography with a solvent system, chloroform–methanol–acetic acid (65:25:10, by vol.). The chromatogram was visualized with iodine vapor; sample no.1 is phosphatidylserine.

phatidylglycerol, sphingomyelin, cholesterol and fatty acids (saturated and monoenoic) did not show hemagglutinating activity. Cardiolipin and phosphatidylglycerol had hemolytic activity.

4. DISCUSSION

Concerning the mechanism of hemagglutination by phosphatidylserine, two observations suggest hydrophobic binding between erythrocytes and the aminolipid. The first is that phosphatidylserine missing 1 mol esterified fatty acid from its molecule did not exhibit hemagglutinating activity at all but did have hemolytic activity. The second is that the hemagglutination was inhibited by phosphatidylcholine and sphingomyelin. The same observations were also performed in an ornithine-containing lipid of *B. pertussis*. As phosphatidylethanolamine, a decarboxylated derivative of phosphatidylserine, did not exhibit hemagglutinating activity, the carboxyl group of phosphatidylserine is presumed to be indispensable in hemagglutination. Inhibition of the hemagglutination by basic amino acids probably indicates their interaction with the carboxyl group of the aminolipid. Further, interaction between sialic acid, aspartic acid or glutamic acid and the amino group of the aminolipid is suggested. In contrast to the unsatisfactory dispersion of an ornithine-containing lipid of *B. pertussis*, phosphatidylserine dispersed very well, causing opalescence. Considering the strong agglutination potency of phosphatidylserine for chicken erythrocytes as shown in fig.1, its 3 binding sites, namely hydrophobic, basic and acidic sites, might be used more effectively, probably as liposome, to combine with erythrocytes than in the ornithine-containing lipids of *B. pertussis*. Anyway, the hemagglutination by these aminolipids was considered to be caused by a mechanism different from that of hemagglutination by lectins.

The hemagglutinating activity of phosphatidylserine may not have been considered worth mentioning up to now, because it is an internal phospholipid in the cell membrane [2] and is an intermediate in the biosynthesis of phosphatidylethanolamine in bacteria [3]. In addition, the activity of phosphatidylserine may have escaped our notice until now, since it is lost easily by sonication. Recently, phosphatidylserine has attracted attention because of its ability to enhance histamine release from mast cells caused by concanavalin A and other substances [4,5]. Mitogenic activity of acidic phospholipids including phosphatidylserine for mouse lymphocytes was reported by Oka et al. at the 1981 annual meeting of the Japanese Society for Bacteriology. Phosphatidylserine may have some specific functions.

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