BIOPHYSICS AND BIOCHEMISTRY

Separation of Peripheral Polypeptides from Soybean Lipid Extracts and Their Effects on Structural Organization of Phospholipids

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Fluorescence assay of soybean lipid extract showed the presence of polypeptides with tryptophan residues in hydrophobic and polar microenvironment. The peripheral polypeptide with polar tryptophan can be separated from phospholipids in solution with high ionic strength. ^{31}P nuclear magnetic resonance spectroscopy demonstrated that this polypeptide stabilized the phospholipid bilayer.

Key Words: *soybean phospholipids; polypeptides; fluorescence; magnetic resonance spectroscopy*

According to the fluid mosaic model, biological membranes consist of noncovalently bound assembles of lipid and protein molecules. Lipid bilayer, the main structural moiety of biological membrane, can be modified by external factors and due to changes in the interaction between its components [8]. The strength of protein-phospholipid bonds in membranes depends on the character of their interaction: peripheral proteins electrostatically bound to the lipid bilayer are separated after increasing the ionic strength, while integral proteins can be solubilized with detergents.

Here we separated peripheral polypeptides from the soybean lipid extract and studied their effects on structural organization of phospholipids.

MATERIALS AND METHODS

Phosphatidylcholine (PC) and phosphatidylinositol from soybean (PI, Sigma) and dipalmitoyl phosphatidylethanolamine (PE, Sigma) were used as tracers.

Soybean lipid extract was obtained from soybean flour (Assoya) by extraction with organic solvents [3], separated by thin-layer chromatography in the following systems: chloroform—methanol—water (65:25:4, system A) and n-butanol—acetic acid—water (4:1:1, system B) on Sorbton-diol plates (MVP Khromdet-Ecologiya) and the spots were visualized with molybdenum blue (phosphorus-containing substances), ninhydrin acetone solution (amino-containing substances), α-naphthol (compounds containing sugar residues), vanillin reagent with α-naphthol (saponins), and vanillin reagent (sapogenins) [4].

The total content of phospholipids in the lipid extract was estimated by the content of phosphorus [11]. Phospholipid fractions were quantified by the corresponding peaks of high-resolution ^{31}P-nuclear magnetic resonance (^{31}P-NMR) spectra recorded on an AM-360 spectrometer (Bruker) at 145.8 MHz for ^{31}P [5]. Broad-line ^{31}P-NMR spectra were recorded on an MSL-200 pulse Fourier spectrometer (Bruker) at 81.01 MHz and broad-line proton decoupling. Fluorescence spectra were measured on a MPF fluorometer (Hitachi) at excitation wavelength of 295 nm and excitation and emission aperture of 3 nm.

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Polypeptide content was estimated by the Bradford method [6] and modified method of Lowry [10] taking into account the concentration of phospholipids in the probe. Bovine serum albumin (Calbiochem) was used as the standard. The polypeptide separated from phospholipids was analyzed in SDS-polyacrylamide gel (15%) electrophoresis (SDS-PAGE) [9].

RESULTS

The total content of phospholipids in the lipid extract measured by microassay [11] was 60% (Table 1). High-resolution ^{31}P-NMR spectroscopy in the system consisting of chloroform, methanol, and 0.2 M Cs-ethylenediamine tetraacetic acid (1:1:0.2) [5] showed that PC, PE, phosphatidylglycerol, and PI were the major components of the preparation (31, 33, 24, and 12%, respectively).

Thin-layer chromatography in system A and staining with specific reagents demonstrated that soybean lipid extract contained not only phospholipids, but also neutral lipids, fatty acids, glycolipids, saponins, sapogenins, and amino-containing compounds belonging to polypeptides.

Fluorescence assay confirmed the presence of polypeptides in the lipid extract. The fluorescence spectrum of water dispersion of 0.2 mg/ml soybean lipid extract had 2 maxima at 330 and 350 nm, which corresponded to tryptophan emission spectrum in hydrophobic and polar microenvironment (Fig. 1, *a*) [1].

The method of Bradford and modified method of Lowry isually applied for measuring the content of polypeptides in membrane preparations. It is believed that phospholipids do not interfere these analyses. Our experiments showed that 0.2 and 1 mg PC had no effect on adsorption of Coomassie brilliant blue G-250 and Folin-Ciocalteu phenol reagent, respectively. In further measurements phospholipid concentrations did not exceed these levels. Protein contents in the lipid extract measured by Bradford and Lowry methods were 7 and 4.5%, respectively. The polypeptide/phospholipid ratio in the lipid extract estimated by the method of Lowry was 1:13. Coomassie brilliant blue

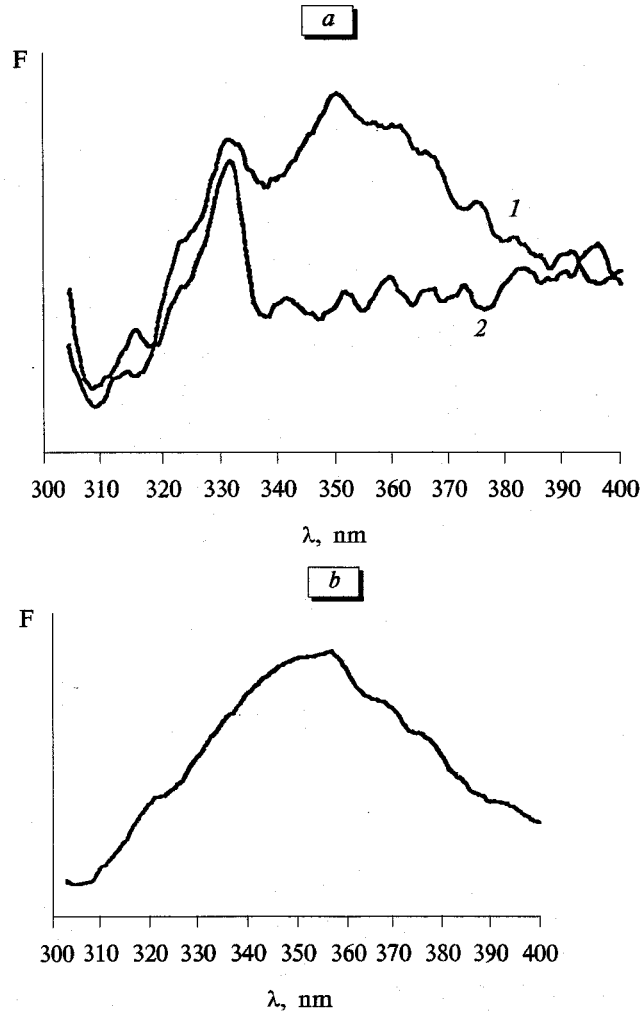

Fig. 1. Fluorescence spectra of water dispersions of soybean lipid extract: *a*) before (*1*) and after (*2*) treatment with 0.1 M NaCl, *b*) polypeptide in water-methanol phase.

G-250 changes its color after binding to polypeptides consisting of at least 10 amino acid residues [2]. Therefore, our results indicate that the preparation contains polypeptides or proteins, rather than individual amino acids.

The soybean lipid extract dissolved in the chloroform-methanol system (2:1) was treated with 0.1 M

TABLE 1. Contents of Phospholipids and Polypeptides in Soybean Lipid Extract (*M*±*m*)

Preparation	Content, % of total weight			Phospholipid/ polypeptide ratio
	phospholipids	polypeptide	nonphospholipid admixtures	
Initial	59±4	4.5±0.5	36±3	13:1
Treatment with 0.1 M NaCl				
chloroform phase	57±5	2.2±0.3	3.8±0.5	26:1
water phase	1.0±0.5	2.4±0.5	33±3	2.4:1

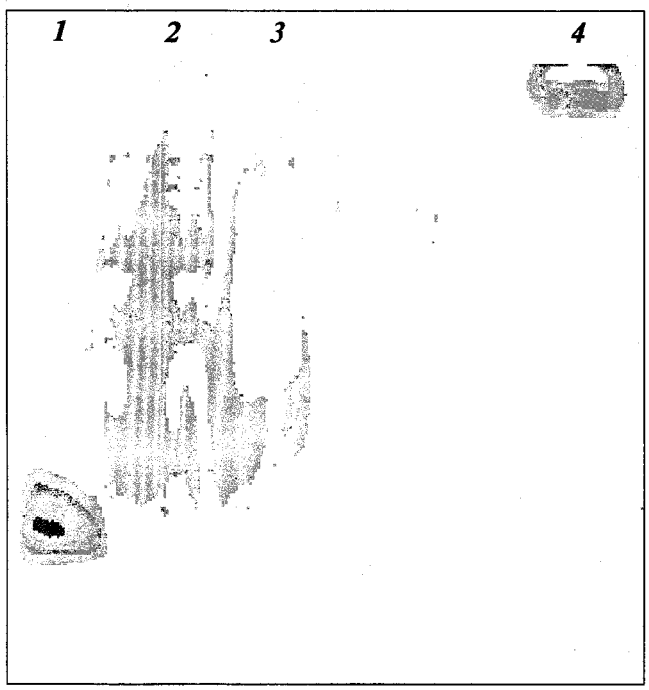

Fig. 2. Gel electrophoresis: soybean inhibitor of proteases of Bowman—Birk type (*1*), initial preparation of extracted phospholipids (*2*), and chloroform phase (*3*) and water-methanol phase (*4*) after treatment with 0.1 M NaCl.

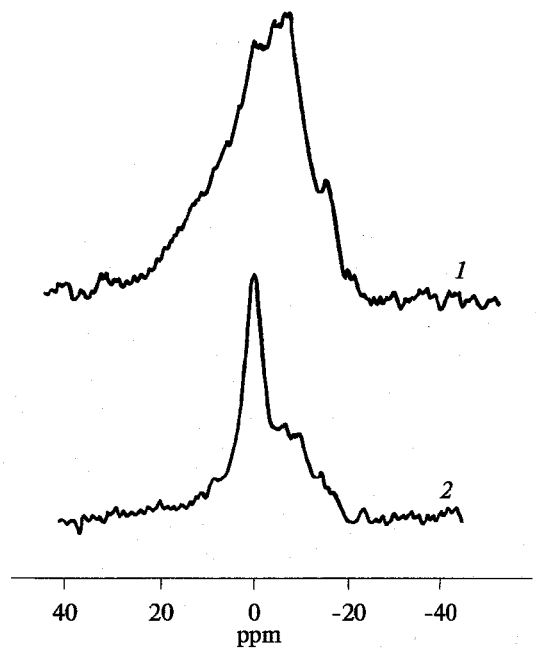

Fig. 3. ^{31}P-NMR spectra of 2% water dispersion of soybean phospholipids before (*1*) and after (*2*) treatment with 0.1 M NaCl.

NaCl for separating peripheral polypeptides. This procedure led to the formation of water-methanol (upper) and chloroform (lower) phases, whose compositions were analyzed by thin-layer chromatography in systems A and B, respectively. All lipid components (neutral lipids, fatty acids, glycolipids, and phospholipids) remained in the chloroform phase, while saponins, sapogenins, and some polypeptides were in the water-methanol phase. The modified method of Lowry showed that a half of polypeptides passed into the water phase and a half remained in the chloroform phase (Table 1). Therefore, the polypeptide/lipid ratio decreased to 1:25. In this case, the method of Bradford was not used because of high concentration of phospholipids in the purified sample.

Before and after the treatment with 0.1 M NaCl, lipid extract was assayed by SDS-PAGE on 2 parallel plates stained with Coomassie R-250 and molybdenum blue to reveal proteins and phosphorus-containing compounds, respectively. SDS-PAGE of the lipid extract revealed the band at the start and diffuse area comprising poorly resolved bands (Fig. 2). A narrow band with low electrophoretic mobility contained only protein with molecular weight surpassing that of control protein, soybean protease inhibitor (8 kDa). The diffuse area was stained with Coomassie R-250 and molybdenum blue indicating the presence of a protein-lipid complex or proteolipid.

SDS-PAGE of the lipid extract treated with 0.1 M NaCl revealed only diffuse band containing proteins and phospholipids. The water-methanol phase included protein with low electrophoretic mobility (Fig. 2).

After evaporation, water-methanol and chloroform phases were dispersed in the water phase. Fluorescence assay showed that the maximum at 350 nm disappeared after the removal of peripheral polypeptides from the lipid extract with 0.1 M NaCl (Fig. 1, *b*). However, the polypeptide with the fluorescence maximum at 350 nm was detected in the water phase (Fig. 1, *c*).

Effects of the polypeptide on the structural organization of phospholipids in water dispersions of preparations were studied by broad line ^{31}P-NMR spectroscopy. Before treatment with 0.1 M NaCl the preparation had a spectrum with 2 maxima at 0 and -14 ppm, which indicated the presence of isotropic and bilayer phases in phospholipid aggregates [7]. After treatment with high-ionic strength solution, the preparation spectrum had only 1 maximum at 0 ppm corresponding to the isotropic phase (Fig. 3). Thus, the bilayer phase is transformed into the isotropic phase after the treatment with 0.1 M NaCl. Destabilization of the bilayer is associated with polypeptide removal, because saponins and sapogenins washed out with this polypeptide act as detergents promoting the formation of nonbilayer structures.

Our studies showed that extraction of soybean flour with organic solvents leads to the formation of a lipid extract containing 2 types of polypeptides (minor components) bound to phospholipids by hydrophobic and electrostatic bonds. The latter polypeptide con-

tains polar tryptophan and can be removed after increasing ionic strength of the medium. This polypeptide stabilizes the phospholipid bilayer in water dispersions.

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