Note

Association of Phosphatidylcholine with Soybean 11S Globulin

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Received August 9, 1982

In the field of food chemistry, protein-lipid interactions are closely related to the texture of foods. We have proved by using spin-labeled phosphatidylcholine (PC)¹⁾ and sucrose density gradient centrifugation methods²⁾ that PC is bound to soybean proteins through both hydrophobic and hydrophilic interactions. In the preceding paper,³⁾ we also found, by using chemical modifying reagents, that PC was not bound to the specific amino acid residues in soybean 7S globulin, while the fluorescence intensity of 8-anilino-1-naphthalene sulfonic acid-modified 7S globulin associated with PC was decreased by increasing PC.

This paper deals with two types of interaction of PC with 11S globulin by using chemical modifying reagents.

Chromatographically purified 11S globulin was prepared from soybeans (Tsurunoko) by the method of Thanh and Shibasaki⁴⁾ and the 11S fraction was used as the protein source. Phosphatidylcholine (4 mg/ml) in 35 mmphosphate buffer containing 0.4 M NaCl (pH 7.6), the standard buffer, was sonicated at 15 sec intervals for 2 min in an ice-bath with a Kaijo Denki T-A-4201 sonicator (20 kHz). The dispersion mixture was centrifuged at $9000 \times g$ for 20 min to sediment any titanium particles and undispersed lipid. The supernatant was stored at 4°C and was used as the PC source. The experimental conditions, interaction of PC with 11S globulin, chemical modifications and the interaction of PC with the chemically modified proteins etc., were according to the methods of the preceding paper.³⁾ All measurements were carried out at room temperature. Phosphorus was determined according to the method of Bartlett.⁵⁾ Protein was determined according to the method of Lowry *et al.*⁶⁾ Radioactivity was measured with a Horiba liquid scintillation spectrometer Model LS-500 in modified Bray's solution: 4 g PPO, 0.1 g POPOP and 75 g naphthalene in 333 ml each of toluene, ethylcellulosolve and dioxane.

Binding of PC to 11S globulin was studied by using radioactive PC. After a mixture of PC and protein was incubated at 37°C for 30 min, it was subjected to chroma-

tography on a Sepharose column which had been equilibrated with standard buffer. The free PC was eluted in the void volume, and then the protein was eluted. The amounts of PC bound to the protein were determined by measuring the radioactivity of the protein eluted. With an increase in the ratio of PC to protein in the incubation mixture, there was parallel increase of the bound PC, as in the case of 7S globulin (Fig. 1).

Mixtures of PC and protein in various molar ratios were incubated at 37°C for 30 min and then subjected to chromatography on a Sepharose 6B column (1.5 × 35 cm). Table I shows the behavior of various amino acid residues of PC-bound 11S globulin. The fluorescent intensity of the eluted protein was measured using the excitation and emission wavelengths of 290 and 340 nm. This change of fluorescence is considered to depend on tryptophan in the protein, and the fluorescent intensity was decreased. The reactivity of tryptophan was also investigated by using 2-hydroxy-5-nitrobenzylbromide (HNBB) at pH 4. The distinct difference in the mole number of the amino acids modified by HNBB was produced by PC.

Behavior of the amino groups of PC-bound 11S globulin was investigated by using reagents for discriminating the state of amino groups in protein. Trinitrobenzenesulfonic acid (TNBS) and 2-methoxy-5-nitrotropone (MNT), which react with amino groups, were used. The mole number of the modified amino groups changed with various molar ratios of PC to protein, and the relationship of the ratio of PC-bound protein to the mole number of the modified amino groups was not linear, but this change may be due to conformational changes of the protein caused by the binding of PC.

Behavior of the hydrophobic region was studied by using the fluorescent probe, 8-anilino-1-naphthalene sulfonic acid (ANS), which reacts specifically with the hydrophobic region of proteins. The fluorescent intensity of the ANS-modified protein was gradually diminished as the ratio of PC to protein was increased.

FIG. 1. Association of PC with 11S Globulin.

TABLE I. BEHAVIOR OF VARIOUS AMINO ACID RESIDUES OF PC-BOUND 11S GLOBULIN

PC: Protein (M: M)	TNBS	MNT	HNBB (pH 4)	Fluorescence intensity (tryptophan)	ANS (512 ~ 513 nm)
	Mole numbers of amino groups		Mole numbers of tryptophan		
0:1	40.5	35.2	9.3	0.53	0.61
46:1	44.6	27.2	5.4	0.49	0.60
77:1	38.1	29.2	6.1	0.48	0.59

Abbreviations: TNBS, trinitrobenzenesulfonic acid; MNT, 2-methoxy-5-nitrotrypone; HNBB, 2-hydroxy-5-nitrobenzylbromide; ANS, 8-anilino-1-naphthalene sulfonic acid.

TABLE II. INTERACTION OF PC WITH PROTEIN MODIFIED BY VARIOUS REAGENTS

PC: Protein (mg: mg)	TNBS	MNT	HNBB (pH 7.6)	ANS (493 ~ 494 nm)
	Mole numbers of amino groups		Mole numbers of tryptophan	
0:1	11.4	20.8	4.4	0.31
1.8:1	11.3	17.6	3.9	0.27
3.0:1	9.8	17.8	3.0	0.27

Table II shows the interaction of PC with 11S globulin modified by various reagents. In the case of soybean 7S globulin modified by various reagents,³⁾ the mole numbers of modified residues were changed by the conformational changes of 7S globulin that were caused by the addition of PC. Interaction of PC with the protein of which amino groups had been modified by TNBS and MNT were investigated. The protein reacted with TNBS and MNT at 37°C for 60 min, respectively. Modified proteins were incubated with various molar ratios of PC, and then subjected to Sepharose column chromatography. Protein fractions were pooled, and the amounts of protein and absorbance at 350 nm (TNBS) and 420 nm (MNT) were determined. The mole numbers of the modified amino groups in various ratios of PC to modified protein were decreased.

Interaction of PC with protein of which tryptophanyl residues had been modified by HNBB was investigated. Determination of the absorbance of HNBB-modified protein was carried out at 410 nm. The numbers of modified tryptophanyl residues per mole of the modified protein were decreased.

Interaction of PC with the hydrophobic region modified by ANS was investigated. The results obtained by using hydrophobic probes suggest that PC is bound to the hydrophobic region of 11S globulin.

From these several lines of evidence, soybean 11S globulin may be considered to be conformationally changed by association with PC, but PC does not bind to specific amino acid residues through either hydrophobic or hydrophilic interactions. It is also confirmed by circular dichroism that the conformation of 11S globulin is changed by the addition of PC, especially that the content of β -structure was decreased, while that of random coil was increased.³⁾

Acknowledgement. The authors wish to thank Miss Y. Yamashita for her technical assistance.

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