Isolation and Characterization of the Multiple 7S Globulins of Soybean Proteins

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ABSTRACT
Two major proteins (the 7S and 11S globulins) of soybean (Glycine max) were simultaneously isolated by a simple method based on their different solubilities in dilute tris (hydroxymethyl)aminomethane buffers. The purified 7S globulins, which represented essentially the entire 7S soybean protein fraction capable of dimerization at 0.1 ionic strength, were fractionated into five components by diethylaminoethyl Sephadex A-50 column chromatography. The five 7S components were characterized by disc-electrophoresis.

The two major reserve protein fractions of soybean seeds, 7S and 11S globulins, have been isolated and characterized by a number of authors (3, 5, 9, 10, 12, 13, 15). However, there have been conflicting reports on the relative electrophoretic mobility of these two fractions in disc gels (1, 2, 7). Even though the 7S fraction of the water-extractable soybean proteins was proved to be composed of at least four components which possessed the sedimentation coefficient of approximately 7S (6), the isolated main 7S globulin capable of dimerization at 0.1 ionic strength was demonstrated to be homogeneous by disc-electrophoresis, gel filtration, hydroxylapatite chromatography, immunodiffusion, and immunoelctrophoresis (11, 12). In recent studies, Hill and Breidenbach (7) isolated the major soybean proteins by sucrose density gradient sedimentation and indicated that the major 7S protein which undergoes a shift in sedimentation properties at low ionic strength exhibits three major electrophoretic bands by disc-electrophoresis. These three 7S components accumulate at apparently different rates during seed development (8).

This report describes a simple, large-scale separation method of the entire 7S and 11S protein fractions of soybean seeds and the isolation of five major components from the 7S fraction by ion-exchange chromatography.

MATERIALS AND METHODS
Soybean Material. Soybean (Glycine max var. Raiden) of the 1973 crop in Iwanuma, Miyagi, Japan was crushed, ground, and defatted through a sieve (60 mesh), and defatted with petroleum ether (b.p. = 30–60°C).

Sephrose 6B Gel Chromatography. The crude 7S fraction (1500 mg) prepared by the method shown in Figure 1 was dissolved in 40 ml of the standard buffer (35 mm phosphate; 0.4 m NaCl; 10 mm β-mercaptoethanol; pH 7.6) and applied to a 4 × 140 cm Sepharose 6B column previously equilibrated with the standard buffer. Fractions of 20 ml were collected and absorbance at 280 nm was recorded.

The protein in pooled fractions was concentrated to about 40 ml by ultrafiltration and rechromatographed on the same column.

DEAE-Sephadex A-50 Chromatography. Fractionation of the multiple 7S globulins was carried out on a 1.5 × 90 cm column of DEAE-Sephadex A-50 equilibrated with pH 7.8 phosphate buffer (16 mm K2HPO4; 3 mm KH2PO4; 10 mm β-mercaptoethanol) made to 0.20 m in NaCl. The purified 7S fraction (200 mg), dissolved in 10 ml of the starting buffer, was applied to the column. The column was developed with 400 ml of buffer before application of the gradient. Elution with NaCl in gradient concentration of 0.20 m to 0.40 m was carried out using a mixer chamber (containing 1000 ml of starting buffer) and a reservoir chamber (containing an equal volume of the phosphate buffer made to 0.40 m in NaCl, pH 7.8). Column effluents were collected in 10-ml fractions and monitored at 280 nm.

Disc Electrophoresis. The modified slab disc-electrophoresis method of Davis (4) and Ornstein (14), which allows simultaneous analysis of up to 11 samples, was used. The details of the modification will be published elsewhere.

RESULTS
Simultaneous Isolation of the 7S and 11S Fractions. The two major soybean proteins were simultaneously isolated by a simple method based on the solubilities of the fractions in dilute tris buffers (Fig. 1).

Figure 2 shows the ultracentrifugal patterns of the crude 7S and 11S fractions prepared by the method. Only a very small amount of the 7S remained in the crude 11S fraction (Fig. 2B) and the crude 7S fraction I was nearly devoid of the 11S (Fig. 2A). About two-thirds of the buffer-extractable proteins were found in the crude 7S fraction I.

The 7S fraction II was further purified on a Sepharose 6B column (Fig. 3) in order to eliminate completely the 2S fraction. Ultracentrifugal patterns shown in Figure 4 indicate that the purified 7S fraction II underwent complete dimerization (conversion into 9S sedimenting form) at low ionic strength. The yields of the purified 7S fraction were on the order of 23 to 25% of the total extractable proteins, or 77 to 83% of the total 7S present initially (assuming that 7S fraction makes up 30% of the total proteins). These are the highest yields that have ever been reported.

Fractionation of the Multiple 7S Globulins. The purified 7S fraction was fractionated into at least five components by DEAE-Sephadex A-50 chromatography as shown in Figure 5. The five 7S components were further characterized by disc-
electrophoresis. Five distinguishable bands were detected. They were numbered consecutively in order of increasing relative mobility (Fig. 6).

As seen in Figures 5 and 6, component 1 first emerged from a DEAE-Sephadex A-50 column; then, components 5, 3, 4, and 2 emerged successively. Component 5, showing a relatively broad band by disc electrophoresis, was not separated from the two near bands (components 3 and 4) when the unfractionated 7S was analyzed (Fig. 6B). The five purified 7S components had the same electrophoretic mobilities as the purified 7S fraction (Fig. 6B) and the 7S protein-bands of the soybean globulins (Fig. 6A). This indicates that no variations in the electrophoretic behaviors of the 7S components occurred during isolation and fractionation processes. Therefore, the isolated components represent the five major 7S components of soybean proteins (judging from the chromatographic elution in Fig. 5 and the dry weight of proteins recovered from the pooled fractions).

### DISCUSSION

Our separation method of the 7S and 11S soybean protein fractions according to their different solubilities in 63 mM tris-HCl buffer (pH 6.6) at 2 to 3°C is a significant improvement.

![Figure 3](image3.png)

**Fig. 3.** Purification of the 7S fraction by Sepharose 6B gel chromatography. A: Gel chromatography of 1500 mg of the crude 7S fraction II; B: rechromatography. Column size: 4 × 140 cm; buffer: standard phosphate buffer (μ = 0.5); fraction volume: 20 ml; flow rate: 50 ml/hr.

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![Figure 2](image2.png)

**Fig. 2.** Ultracentrifugal patterns of the crude 7S fraction I (A) and crude 11S fraction (B). The protein concentration was 0.9% in the standard phosphate buffer (μ = 0.5). Photographs were taken after 52 min of centrifugation at 55,430 rpm at 70° bar angle. Direction of sedimentation is from left to right.

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**Fig. 1.** Schematic outline of the simultaneous separation of the 7S and 11S fractions from defatted soybean meal. Buffer 1: 63 mM tris-HCl buffer containing 10 mM β-mercaptoethanol, pH 7.8; buffer 2: 63 mM tris-HCl buffer containing 10 mM β-mercaptoethanol, pH 6.6, at 2 to 3°C.

**Defatted Soybean Meal**

- Extract with Tris-HCl buffer¹ (Meal:buffer, 1:15) for 1 hr
- Centrifuge, 10,000 rpm, 15 min

**Whole Buffer Extract**

- Add 2N HCl to pH 6.6
- Dialyze against Tris-HCl buffer²
- at 2 to 3°C for 3 hr
- Centrifuge, 10,000 rpm, 20 min

**Precipitate**

**Crude 11S Fraction**

**Crude 7S Fraction I**

**Precipitate**

**Crude 7S Fraction II**

**Precipitate**

**Crude 7S Fraction**

**Purified 7S Fraction**

**Supernatant**

**Crude 11S Fraction**

**Crude 7S Fraction I**

**Supernatant**

**Crude 7S Fraction II**

**Supernatant**

**Whey Proteins**

**Crude 7S Fraction I**

**Whey Proteins**

**Crude 7S Fraction II**

**Whey Proteins**

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**Legend:**

- **Figure 1:** Schematic outline of the simultaneous separation of the 7S and 11S fractions from defatted soybean meal. Buffer 1: 63 mM tris-HCl buffer containing 10 mM β-mercaptoethanol, pH 7.8; buffer 2: 63 mM tris-HCl buffer containing 10 mM β-mercaptoethanol, pH 6.6, at 2 to 3°C.

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**Figure 3:** Purification of the 7S fraction by Sepharose 6B gel chromatography. A: Gel chromatography of 1500 mg of the crude 7S fraction II; B: rechromatography. Column size: 4 × 140 cm; buffer: standard phosphate buffer (μ = 0.5); fraction volume: 20 ml; flow rate: 50 ml/hr.
over other reported methods (10, 12, 15) both in yield and the simplicity of procedure. This method, which can be used for large scale preparations, requires only a few hours and results in the quantitative recovery of the two fractions with very little cross-contamination. Quantitative recovery of the 7S fraction by this method ensures that all of the individual components are present. One reason for the conflicting data reported by several authors is the purification method of the 7S fraction by gel chromatography in order to eliminate the contaminated 11S (10). Our studies (16) had indicated that this method would result in the loss of some of the major 7S components.

By our method, approximately 80% of the total 7S fraction was isolated from buffer-extractable soybean proteins. The other 20% of the initial 7S may be fraction that does not dimerize at low ionic strength (18). Our method was also used successfully to prepare the 7S and 11S fractions from immature soybean seeds (K. Uno, K. Okubo, and K. Shibasaki, unpublished data). Thus, this method may be appropriate to investigate the accumulation of the major soybean globulins during seed development.

The presence of multiple major 7S components in soybeans has only recently been recognized. Using disc-electrophoresis, Hill and Breidenbach (8) have indicated that the three electrophoretic components of the 7S fraction accumulate at different rates in immature soybean. Our previous studies (16) had suggested that the major 7S globulins of soybean consisted of at least five electrophoretic components that were distributed in a distinct order by gel chromatography. In the present work, the five components were isolated by ion-exchange chromatography. The length of the column and the nature of the gradient seem to be the predominant factors for the resolution. Of the five components, component 5 was not clearly separated from component 3 and component 4 by disc-electrophoresis. The four components (component 2 to 5) probably correspond to the three major 7S bands detected by Hill and Breidenbach (7, 8). We have no explanation for the existence of component 1. Possibly this is attributable to different variety of soybean used in our experiments.

Our preliminary results indicate that the five 7S components are composed of four major subunits (separated by polyacrylamide gel electrophoresis in a system containing 5 M urea, 30%
acetic acid) in different ratios. This may suggest that they are not unrelated to each other, but accumulate at different rates during biosynthetic process.

All the five components are glycoproteins (V. H. Thanh and K. Shibasaki, in preparation) which differ from the 11S component (16). The relations of the heterogeneity reported here and that of the carbohydrate units of the 7S soybean globulins (19), and the significance of the glycosylation (17) of the 7S protein are important subjects for further investigations.

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LITERATURE CITED